Pharmacogenetic Investigations Using Community-Based Participatory Research to Address Health Disparities in Minnesota Hmong

A Dissertation

SUBMITTED TO THE FACULTY OF

UNIVERSITY OF MINNESOTA

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

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August 2017

Acknowledgements

This dissertation would not have been completed unless I had the support from a multitude of individuals from the Experimental and Clinical Pharmacology (ECP) Department and the College of Pharmacy at the University of Minnesota. First and foremost, I'm beyond grateful to my advisor, Dr. Robert Straka, for all his support, guidance and patience that he offered me throughout my training. Looking back on where I started and who I'm today, professionally, I realize that my career development was the result of continuous feedback and ongoing assessment of my work during the graduate school.

I also want to thank Dr. Kathleen Culhane-Pera for being an outstanding community collaborator and support to my success. Indeed, she is an example to look up to when working with special and underserved communities.

Finally, I want to thank the Hmong board members for their important role in making the university-community partnership very successful, the GOUT-H study volunteers, the study participants and the ECP faculty and staff.

Dedication

This dissertation is dedicated to my dad and grandmother whom I miss dearly.

Abstract

Introduction: Pharmacogenomics is an approach to personalizing therapy to help patients achieve their therapeutic goals with the least possible adverse events. This approach relies on the knowledge derived from large genetic studies that involve diverse populations to guide the development of treatment algorithms. The underrepresentation of select populations or unique sub-populations in genetic-based research presents as a gap in knowledge to create comprehensive genetic-based treatment algorithms and a missed opportunity to address health disparities within those unique populations. A prime example is the Minnesota Hmong. The Hmong is an Asian sub-population minimally represented in clinical or genetic-based research with a high prevalence of gout and gout-related comorbidities than non-Hmong.

Methods: Using the principles of community-based participatory research and the establishment of the Hmong advisory board, assessment of the community's perception of genetics and preparedness for engagement in research were conducted. Capitalizing on the findings from the first informational study, two Hmong genetic-based studies were conducted. The first study was to ascertain the frequency of select pharmacogenes and disease-risk genes in the Hmong, relative to non-Hmong. The second study was to quantify the effect of genetic variations within uric acid transportome and purine metabolizing genes on the pharmacokinetics and pharmacodynamics of allopurinol in Hmong adults with gout or hyperuricemia.

Results: The informational study results indicated that most Hmong are willing to participate in research to help themselves and the Hmong community. Some of the genetic perceptions in the Hmong were not scientifically grounded and some concerns about privacy were reported while the return of genetic results to participants had mixed responses. The first genetic-based study indicated that more than 80% of Hmong participants were willing to store their DNA for future analyses and share their DNA with other scientists. Pharmacogenes risk allele frequencies of *CYP2C19, CYP2C9, VKORC1,* and *CYP4F2* were higher in the Hmong relative to Caucasian. Disease risk allele frequencies of hyperuricemia and gout associated genes such as *SLC2A9,* SLC17A1, *SLC22A11, SLC22A12, ABCG2, PDZK1*, were also higher in the Hmong than Caucasian and Han-Chinese. The second genetic-based study indicated that the genetic variation within *SLC22A12* (rs505803T>C) significantly affects the exposure to and the renal clearance of the active metabolite of allopurinol, oxipurinol. Additionally, the rs505802 was also significantly associated with the overall response to allopurinol.

Conclusions: Engaging the Hmong in genetic-based research is a step forward to advance precision medicine while addressing health disparities within the Hmong community. The prevalence of pharmacogenes within the Hmong suggest that the Hmong will require a lower starting dose of warfarin and unlikely to benefit from clopidogrel. The prevalence of hyperuricemia and gout associated risk alleles in the Hmong are consistent with the higher prevalence of gout in the Hmong. Finally, the rs505802 T>C within SLC22A12 gene could predict the overall response to allopurinol.

Table of Contents

List of Tables

List of Figures

Chapter 1

Review Article

Perspectives on Health Disparities of Gout within the Minnesota Hmong and the Opportunities for Genetic-Based Investigational Studies

Chapter Overview

The purpose of this chapter is to give a brief introduction to the history and culture of the Hmong. The chapter also highlights some key health disparities within the Hmong, primarily gout and gout-related comorbidities. Additionally, the chapter explores some barriers associated with engaging minorities in clinical and genetic-based research. Moreover, the chapter presents key principles for conducting clinical and genetic-based research in minority groups. The chapter concludes with an example of building a community engagement research group to ultimately reduce ongoing health disparities within the Minnesota Hmong and potential research opportunities within this unique population.

The History and Demographics of the Hmong

The Hmong are a distinct Asian ancestral group originated from the southern parts of China and migrated to mountainous areas of Laos, Thailand, Vietnam, and Cambodia. In spite of their continuous migration, many of the Hmong maintained their agrarian lifestyles in their respective geographical areas.[1] During the Vietnam War, the Hmong became allies with the United States, which ultimately facilitated many Hmong refugees to resettle in the United States at the conclusion of the Vietnam War.[2] The Hmong population in the US has grown since 1975 with a 40% increase from 2000 to 2010. Per the 2010 US Census, the Hmong number more than 260,000. The Hmong population is not equally distributed across the US, rather it is clustered in three major states. The three largest Hmong communities in the US, in a descending order, are California (91,224), Minnesota (66,181), and Wisconsin (49,240). The metropolitan areas with the highest

number of Hmong, in a descending order, are Minneapolis-Saint Paul, MN; Sacramento and Fresno, CA; and Milwaukee, WI. [3]

The Hmong society is kinship-based and places tremendous emphasis on lineage and clan ties for security and mutual assistance. Making a medical decision within the Hmong is a communal act rather than an individual choice.[4] To illustrate, in medical crises or serious illness, the individual could travel long distances to consult with other members or the leader of the clan to make such a decision. In addition, the Hmong beliefs also play a major role in their health-related decisions. As an example, many of the Hmong are hesitant about the effectiveness of western medicine and tend to opt out of the western medicine because it contradicts with their beliefs.[1] One of the predominant belief systems in the Hmong is the Animism (Shamanism) religion. The followers of this religion believe that illness or sickness of the body is due to a separation or loss of one's soul. This belief system can in part explain the rationale of why a high percentage of Hmong tend to be reluctant to donate blood or undergo certain types of surgeries that can result in organ removal or loss. Nonetheless, engaging in soul calling worship is a major practice for healing among the Hmong who are experiencing an illness or disease. [2] However, these beliefs and practices tend to be greatly influenced by the elderly, newly arrived into the US, and the less educated. Although the health belief system within the Hmong is on a continuum scale, this gradient within their belief system has substantially influenced the health of Hmong community, perception of medical care, and reception and engagement in clinical research.

The Hmong also present with a high and differential prevalence of major disease

risk factors as well as certain disease states compared to other ethnic groups, mainly Caucasians. Anecdotal reports and primary research suggest higher incidence of gout, obesity, diabetes, hypertension, hepatitis, and select types of cancer in the Hmong community compared to non-Hmong or non-Hispanic whites.[2,5-7] Nonetheless, there is very limited systematically-collected information on the prevalence of these diseases in the US vs. their homeland and epidemiological research comparing the development of these diseases in their new environments vs. their native homeland. This lack of data could be attributed to the continuous migration lifestyle of the Hmong while living in their native lands, resettlements in refugee camps after the Vietnam War, and eventually immigrating to the US. In the absence of such data allows us to propose the role of two theorems that could explain the differential prevalence of those health conditions between Hmong and non-Hmong.

The first theory is the Epigenetic Theory of Change, which involves the role of exposure to different environmental factors that could modulate the levels of expression of certain genes. Thus, the change of environment could ultimately modulate the person's disease risk. The second theory is the Paradoxical Theory of Change by Gestalt, *the change that occurs when one becomes what he is, not when he tries to become what he is not,* may be relevant to the Hmong's situation in that behavioral changes and acculturation due to change of environment could influence the person's disease risk. By combining the two theorems, we suggest that adapting to a western diet and lifestyle could have an impact on the onset of the disease or unmask the individual's propensity to develop certain diseases. However, data on the prevalence of these disease states in the Hmong's

homeland is needed to cement this rationale.

Gout Prevalence across Racial Groups

Gout is the most common inflammatory arthritis and is caused by the deposition of monosodium urate crystals in the distal joints. The formation of monosodium urate crystals is generally preceded by chronic elevation of serum uric acid levels also known as hyperuricemia. The global prevalence of gout is roughly 0.1%.[8] However, the accurate estimation of gout prevalence in certain countries or racial groups can greatly differ owing to variations in the methodologies used in in those studies. These limitations include failure to qualify such estimates across sex, age bands, different case definition of gout and calendar year. Those studies reporting the prevalence of gout relying on "self-report" are also prone to recall bias. Furthermore, the classifications of race and ethnicity may also confound the interpretations of gout prevalence, especially in a high admixture population where self-report of race or ethnicity is imprecise. Irrespective of the inconsistency of the methodologies used in different studies, the Oceanian countries particularly with ethnic groups such as Taiwanese aboriginals and Maori remain to have the highest prevalence of gout reported in which some estimates in excess of 10%.[9] The notable high prevalence of gout in these populations may be attributable to a cultural specific diet and lifestyle as well as genetic predispositions for hyperuricemia or gout.

In Europe, Greece has the highest prevalence of gout with an estimate of 4.75% while the prevalence of gout in the UK is 2.49% of the entire population (Figure 1.1).[10] In contrast, gout is reportedly rare in former Soviet Union regions, Guatemala, Iran, Malaysia, and Philippines. In North America, the prevalence of gout in the US was

estimated to be 3.9% of adults.[11] Although not statistically significant, the data from the Third National Health and Nutrition Examination Survey (NHANES) 2007-2008 showed that blacks have a higher prevalence of gout with 5.0% compared to whites with 3.9%. In Japan, although lower relative to other populations, the prevalence of hyperuricemia and gout have been shown to be trending upward which parallels the upward trend in the utilization of urate lowering therapies. Specifically, the prevalence of gout among Japanese men has nearly doubled from 0.86% to 1.67% from 1969 to 2003.[12,13] This dramatic increase of gout prevalence amongst Japanese should be also assessed in the context of other trends that could contribute to new onset of gout such as the increased prevalence of western diet and obesity. In fact, a 3 to 4-fold increase in obesity rates in Japanese women and men were reported from 1962 to 2002, which could greatly explain the twofold increase in gout prevalence. [14]

These upward trends across different populations are consistent with the global increase of hyperuricemia and gout. However, this increase of prevalence of these two conditions should not be considered independent of other comorbidities and dietary habits that all of which can significantly contribute to the development of hyperuricemia and gout. For example, the increase of prevalence of obesity, chronic kidney disease, hypertension, and the use of drugs that treat these conditions are important confounders for this global phenomenon. In addition, the global consumption of high fructose corn syrup and alcohol are also important contributors to this global prevalence of gout and hyperuricemia.[15,16] The focus of this chapter, however, will concentrate on the impact of dietary and lifestyle choices on the risk of developing gout.

Gout Prevalence in Minnesota Hmong

The prevalence of gout in the Minnesota Hmong was estimated by two methods. These included self-reported gout and physician-diagnosed gout. The self-reported gout prevalence among the Minnesota Hmong was estimated from two cross-sectional community surveys then compared to the national data provided by NHANES III. In addition, physician-diagnosed gout was also estimated from Hmong residents in the Twin Cities from 11 primary care clinics. Findings from self-reported gout among the Minnesota Hmong (Figure 1.2) indicated that there was a 2-fold higher prevalence than the average US population reported in the NHANES III (6.5 % vs. 2.9%; $p < 0.001$). While Hmong women reported having gout at a rate like non-Hmong women (1.9%), Hmong men were significantly more than likely to report gout than non-Hmong counterparts (11.5% vs. 4.1%; $p<0.001$).[17]Similarly, physician-diagnosed gout indicated that Hmong men were more than twice as likely compared to non-Hmong men seen at the same primary care clinics (6.1% vs. 2.5%, $p<0.001$), while Hmong women were equally as likely to be diagnosed with gout as non-Hmong women (0.8 % vs. 0.7%; $p=0.833$).[17]

Gout is generally associated with other comorbidities that underscore the importance of optimal management of gout. In general, patients with gout generally present with multiple comorbidities that could be directly associated with acutely elevated SUA such as uric acid kidney stone or uric acid nephropathy.[18] Other conditions associated with chronically elevated SUA could include hypertension, dyslipidemia, obesity, kidney disease and metabolic syndrome.[11,18-20] The mechanisms by which chronic hyperuricemia or gout affect the development of cardiovascular diseases have been linked to decreased

production of endothelial nitric oxide, activation of the renin-angiotensin-aldosterone system, decrease insulin sensitivity, increased intra-glomerular pressure and glomerular sclerosis.[21-23]

The high prevalence of gout among the Minnesota Hmong is also associated with other clinical manifestations such as uric acid kidney stones. A retrospective chart review of Hmong adults in a large urology group practice in the Twin Cities, 86 Hmong patients with kidney stones were compared to 86 non-Hmong patients with kidney stone of similar age, sex. Out of the 86 Hmong kidney stone patients, 24% had staghorn calculi vs. 0% in the non-Hmong patients with kidney stones. The surgical management of these calculi required more invasive treatment such as percutaneous nephrolithotomy, nephrectomy compared to non-Hmong. The composition of kidney stones was available for 40 Hmong and 39 non-Hmong. Stones with 100% uric acid were found in 35% and 5% of stones of Hmong and non-Hmong, respectively ($P<0.001$). The study concluded that a Hmong patient coming to a urologic attention is likely to be younger with four-fold higher risk for a stone disease than a non-Hmong patient counterpart. In addition, Hmong patients with stones were five-fold higher to have uric acid calculi compared to non-Hmong patients.[24] Collectively, these observations highlight the health burden of gout and gout-related comorbidities on the Hmong while being also associated with high direct and indirect healthcare cost.

Hmong Perceptions of Managing Chronic Diseases

The health belief model including the theory of Reasoned Action provides a framework to identify the underlying basis for certain health related behaviors in minority

populations. According to *Barret et al* [25] Hmong patients in general do not want to hear about the risks or the negative outcomes associated with a long-term treatment or the negative outcomes associated with unmanaged chronic diseases. This health belief model could explain why Hmong patients are more likely to seek treatment of acute symptomatic illnesses as opposed to chronic disease. This health-related behavior could well support the anecdotal reports that Hmong patients with gout tend to have a lower adherence rate to and suboptimal use of allopurinol compared to Caucasians.[26] Hmong patients with gout are more than likely to self-medicate to seek relief from acute gout flares rather than follow a plan that potentially "cures" gout. This tendency is common in many Hmong patients due to their perceived lack of efficacy of the current Western treatment approaches and the rise of complementary and alternative medicinal (CAM) approaches - especially in rheumatic and musculoskeletal diseases.[27]

It is important that clinicians are cognizant of their patients' use of CAM to tailor the best approach for managing that individual's condition. The use of CAM has been attributed to several reasons that are worthy of elaboration. These reasons are categorized into two domains. The first includes access to healthcare and timeliness to obtain symptoms resolution. Relying on a clinician-controlled decision can be ineffective in times of new or acute onset of pain. Consequently, having access to some treatment or therapy that promises timely relief may be in the best interest to the patient.[27,28] The second domain includes patient's autonomy and the holistic belief system. Many patients utilize CAM due to their health or spiritual belief system.[27,28] Several factors that promote patient's autonomy are seen by the patient's ability to have access to a wealth of health

information through health-based apps, direct-to-consumers testing, online websites, social media and access to products that promise relief and comfort. Other reasons may include sub-optimal physician-patient relationship or mistrust in the western medicine due to certain cultural beliefs or a negative treatment experience with poor outcomes.[1]

As an example, *Lor et al* [29] provided a closer look at the frequency and perception of herbal medicinal use among Hmong-American using a telephone-based survey. The method used to determine eligibility was the surname of 18 clans. Participants had to be 18 or older and with a partial or Hmong descent. The study concluded that Hmong-Americans of Sacramento, CA have a higher CAM use relative to other ethnic groups reported by the by 2002 National Health Interview Survey Alternative Medicine Supplement. In fact, out of 118 interviewed participants 77(65%) reported using medicinal herbs. Although the study had many limitations, the results provided are consistent with what has been previously reported on Hmong's perception of western medicine.[1] The use of medicinal herbs, however, seem to be associated with certain demographics. These factors include being a male, having less formal educational level, and being born outside the US. [29] The high utilization of herbal and nonwestern medicine among the Hmong represents a critical step towards providing optimal patient care. Specifically, the concomitant use of herbal or non-herbal supplements along with the risk of polypharmacy present a potential perfect storm of risk for major drug adverse events. Therefore, the knowledge of alternative medicine use among the Hmong can be important in managing patient therapy to avoid drug–drug interactions and drug-disease interactions while optimizing the therapeutic outcomes.

Dietary and Lifestyle Practices of the Hmong and Risk of Gout

Diet and lifestyle are major risk factors for the development of gout.[10,30-32] Specifically, low- energy expenditure lifestyle and diet high in purine content in conjunction with declining kidney function represent an elevated risk for developing new onset of gout. Historically, the Hmong from Laos and other parts of China have been known for their agrarian lifestyles that allowed them to be physically active and rely on food resources that are either produced or raised in their local communities. Although the pace of adapting to western diet can vary in speed within the Hmong culture, the immigration to the US has evidently and significantly reduced the high-energy expenditure associated activities. This change in the lifestyle and adaption to a western diet could, in part, explain some of the findings presented in the surveys and interviews of the Hmong living in California.[33]

Yang and Mills [33] conducted a survey of 248 Hmong participants living in Fresno, California to assess the pattern of tobacco smoking, alcohol consumption, and dietary practices within the Hmong. The study revealed that more than 63% of Hmong are either overweight or obese (BMI$\geq$25 kg/m^2),[33] which is a risk enhancer for numerous diseases including gout. From a social lifestyle perspective, the Hmong are less likely to excessively smoke tobacco or consume alcohol, which provides some protection against diseases that are caused by alcohol and tobacco use.[33] Specially, high consumption of alcohol can increase uric acid and precipitate acute gout attack. On the other hand, cigarette smoking can reduce uric acid levels as well as the risk of gout. Thus, knowledge of social lifestyles may predict the risk of gout.

While the Hmong were reported as less likely to excessively consume alcohol, this report does not reflect the true estimate of community use of alcohol due to the seasonality and calendar year. Although *Yang and Mill*[33] did not account for these variables in their dietary survey, personal communications with Hmong community members and Hmong key informants[34,35] have highlighted two aspects to alcohol consumption within the Hmong community. First, Hmong social celebratory events (Hmong New Year, weddings) and funerals tend to include high amounts of alcohol consumption. Second, per the Hmong cultural norms, the invited guests to these events are expected to partake in the event by consuming the food and beverages offered to them. In fact, refusal to consume any of the food or beverages could be a sign of disrespect to the host. Therefore, it is possible to speculate that gout patients can easily become socially isolated from the Hmong community due to their gout as they cannot consume some food or alcohol provided to them during these events. This social burden on Hmong patients with gout may lead to other complications that could adversely affect individuals of a high kinship community.

On the dietary side, the Hmong frequently consume a select food items daily such as regular rice (79%), chicken (44%), pork (20%) beef (19%), egg (16%) and fish (7%).[33] On a weekly basis, the Hmong participants reported consuming beef (56%), pork (52%), chicken (47%), egg (45%) and fish (28%). The study also revealed that most Hmong do not consume fruits and vegetables daily but rather on a seasonal basis.[33] For example, apples (8.5%), bananas (6.5%), oranges/tangerines (6.5%) and honeydews (3.2%) were reported to be consumed daily versus 45%, 48%, 48% and 54% on a seasonal basis,

respectively. The consumption of vegetables also paralleled the consumption of fruits by being less daily compared to seasonal basis. For example, sweet peas (2.4%), eggplants (1.6%), and cucumbers (2.4%) and cabbage (4.8%) were reported to be consumed daily versus 54%, 54%, 52%, and 39% on a seasonal basis, respectively. The use of non-alcoholic beverages, carbonated soda, and milk were also assessed in the survey. The study revealed that about 10% of the participants reported drinking cola drinks every day while those reported drinking orange juice and milk were 9% and 6%, respectively.[33] The above observations of patterns of dietary and lifestyle choices (alcohol, smoking sedentary life) in the Hmong are consistent with a population at elevated risk for hyperuricemia and gout.[10,32,36] Although not specifically compared with other ethnic groups, they represent findings that are consistent with others examining dietary patterns that influence uric acid levels and risk for gout.[30,32,36-38]

For instance, *YI-Tsen et al* [39] identified three dietary patterns that can influence uric acid levels known as *uric acid-prone pattern*, *fish and fried food pattern* and *vegetable and fruit pattern*. Specifically, in Chinese adults within Taiwan, the serum uric acid of participants in the *uric acid-prone pattern*, (which included seafood, meat, alcoholic beverages, and organ meat) was significantly higher compared to participants within the vegetables and fruits pattern. In addition, serum uric acid levels trended significantly as the quartiles increased in the *uric acid-prone pattern*.

Additionally, a prospective study evaluating relationship between diet and the risk of gout in Singapore Chinese population identified significate hazard ratios (HRs) of select diets with physician-diagnosed gout.[30] For instance, comparing the first to fourth quantile

of total protein was associated with HR 1.27 [95% CI (1.12-1.44)] of gout. This significant trend was also consistent with the sources of protein such as poultry, fish, and shellfish with HRs 1.27, 1.16, and 1.16, respectively. On the other hand, the study identified that the consumption of soy food and non-soy legumes was significantly associated with reduced risk for developing gout with HRs of 0.86 and 0.83, respectively.

Consequently, the high intake of purine rich protein in general and decreased intake of sources known to mitigate or reduce the risk of gout such as fruits, vegetables, orange juice, and milk all represent optimal conditions for developing gout. These dietary choices which happen to be documented as prevalent in the Hmong, would be expected to enhanced risk for developing gout. While diet and nutrition play key roles in the development of diabetes and obesity, they are also a prominent risk factors for the development of gout. The generational effects of migration, acculturation and assimilation of dietary intake and to the western lifestyles on Hmong include increasing obesity risk among second and third generation compared to those who have recently immigrated.[40] These findings were also reported in the National Longitudinal Study of Adolescent to Adult Health particularly among Asian American and similar studies.[41-43] Therefore, Hmong diet and their unique risk of obesity seem to correlate with the increased risk of gout. Consequently, in-depth understanding of the inherent predisposition to elevated serum uric acid and gout in conjunction with the clinical pharmacology of lowering serum uric acid will provide comprehensive analyses of why the Hmong have a high prevalence of gout and pharmacological means to address a highly prevalent health condition within the Minnesota Hmong. In addition, an appreciation to role of diet and lifestyles may have

on the development of the disease in this unique population, can be used to optimally and comprehensively manage a disease of a growing prevalence within the Hmong.

Hmong and Minorities in Clinical Genetics Research

The recent advancements in technologies and the declining cost of genetic testing have driven an unprecedented growth of genomic discoveries in our recent memories. In the past decade alone, there has been an increase in genetic-based investigations, growing knowledge of the impact of human genetic variations on human health and the development of genetic-based guidelines for drug selection. This genetic revolution and the recognition of the importance of including large number of individuals from different ethnic and cultural backgrounds to donate biological samples has further advanced genetic research.[44,45]

The multiethnic and racial representation including the Hmong in clinical trials and/or clinical genetic research remains limited due to challenges attributed to geographical, cultural, educational barriers as well as mistrust experiences.[1,34,35] Although a substantial effort has been placed to ameliorate these barriers to participate in research within select ethnic and racial groups, mainly African Americans, further work is needed to engage other populations in clinical research.[46] One example is the Asian population. It is evident that within the Asian race there are many sub-populations that are culturally unique and present with different levels of risk for developing select diseases.[2] Consequently, the practice of utilizing aggregate results pertaining to the broad categorization of "Asians" masks the potential differences which likely exist between subgroups within the Asian race. This imprecise approach leads to inaccurate

generalizations and undermines the full potential and value of personalized therapy.

Currently, up to 75% of the Genome-Wide Association Studies (GWAS) are being conducted on European descendants.[47] The results and information from these landmarks genetic studies are serving as the basis for developing the commercial direct-to-consumers genetic testing kits and informing the genetic based guidelines. This lack of inclusivity of other minority groups represents a major shortcoming for the generalizability and usability of these results and applicability of these genetic testing kits across the population at large.[47,48] This lack of knowledge could widen the gap of health disparity across racial groups by creating disproportionate levels of knowledge about select populations. In addition, the lack of replications of major findings in mainstream–based GWAS underestimates the allelic architecture, genetic signatures and major linkage disequilibrium in these minority groups.[48]

Clinical Genetic Research Challenges and Opportunities

Although genetic research continues to rapidly progresses, inclusion of specific racial, multiethnic and sub-ethnic groups in research remain a challenge.[46] The lack of representation of special populations in genetic research has mobilized efforts to raising awareness of the values and benefits of participating in both clinical and genetic-based research and creating the National Institute of Health Policy and Guidelines on the Inclusion of Women and Minorities in Clinical Research[46] as well as tailoring the consent process to accommodate cultural and ethnic specifics to participate in genetic-based research.[49]

There are some well-recognized challenges associated with conducting clinical

research in general, and specifically genetic-based research. These include ethical concerns, privacy issues, sharing genetic information with other investigators, handling incidental findings, processing of variants of unknown significance, and returning genetic data to participants.[50,51] Addressing these challenges requires an adaptive mechanism to facilitate the research process while maintaining an adequate balance of risks and benefits to study participants. Another unique challenge complicating the conduct of genetic-based research is the study of vulnerable populations or minorities. These groups of individuals can present with language and cultural barriers, limited knowledge of genetics, and low literacy levels that may greatly hinder their participation in genetic research. These challenges can ultimately contribute to the development of health disparities of genetic knowledge preventing select communities from benefiting from these scientific endeavors. Consequently, recognizing important core merits of scientific, cultural, and social factors are important to designing ethically responsible approaches to informed consent in genetic-based studies involving ethnically, linguistically, socio-economically diverse population. Table 1.1 provides a summary of core factors, which ensure a successful a genetic-based research in special populations.[49]

While the social and ethical issues associated with the process of informed consent for genomic research remain challenging for participants, community members, investigators, policy makers, and funding agencies have strived to overcome these challenges creating a research model that ameliorates these barriers. For example, the Community-Based Participatory Research (CBPR) paradigm has shown a great promise to mitigate these challenges. The CBPR has been shown to work effectively in reducing

health disparities and targeting specific health problems identified by the community in a vulnerable population. This research model represents the community while collaborating with researchers to address health concerns identified by the community and recruiting participants from the community. This approach empowers the community and creates participatory environment for researchers and community to produce actionable data that can benefit the community. Historically, community based participatory research is defined as a partnership approach that equitably involves all participants in all aspects of the research process where each person shares his/her expertise to enhance knowledge and to develop interventions that will benefit the whole community. However, to conduct a successful and effective CBPR, there are key principles that need to be agreed upon and followed by all individuals involved in the research process (Table 1.2). [52,53]

In addition, three key principles are also proposed to outline the framework for conducting genetic-based research studies within indigenous populations. These principles are 1) while respecting the culture of the community, do not violate basic principles of ethics, including the institutional review board rules and the use of bribery; 2) realize the existence of many local cultures within one seemingly homogeneous group and act accordingly; and 3) make a long-term commitment as one may need and want to return on multiple occasions.

Implementing Community-Based Participatory Research Model in Minnesota Hmong

Background:

The lack of Hmong representation in genetic research, the rising prevalence of chronic diseases within the Hmong, and concerns about growing health disparities

between Hmong and Non-Hmong have alarmed Hmong-treating physicians and university investigators with interest in minorities genetics. This combined interest between the community and academic researchers has led the Westside Healthcare System and the University of Minnesota, College of Pharmacy to build their partnership with the vision of creating a Hmong Genomic Board. The ***long-term goal*** of the board is to help address health disparities between the Hmong and non-Hmong. The ***short-term goal*** is to establish the framework by which the Hmong Genomic Board should function to conduct CBPR. The ***objectives*** of the Hmong Genomic Board were to help the community to identify and address some key health conditions that are greatly affecting a substantive number in the Hmong community while identifying means to increase the Hmong representation in genetic and non-genetic based research.

By facilitating the process of conducting CBPR, the establishment of the Hmong Genomics Board became the first step towards creating health equity between Hmong and non-Hmong. Establishing a Hmong Genomics Board was not without its challenges. These challenges included (1) achieving genuine collaboration, establishing trust, addressing power differences, building equitable relationships to meet the goals of the board; (2) identifying key elements involved in effective collaborative efforts and evaluating the extent to which and how these elements contribute to partnership's success. The following describes the approach used by our group in establishing this board to work toward our common goal.

Approach:

The evolution of the Hmong Genomic Board was led by two prominent leaders who

shared similar interest in addressing health disparities within minority populations in Minnesota. As an experienced primary care physician who works extensively with the Hmong (KCP) partnering with a University of Minnesota College of Pharmacy Professor (RJS) with expertise in pharmacogenomics led to the recognition of forming the Hmong Genomic Board, as the basis for future research endeavors within the Minnesota Hmong. With the vision of conducting prospective CBPR within the Minnesota Hmong, key Hmong community leaders and experts were identified and invited to participate in the newly formed board. Once the identified members agreed to join the board, the purpose, scope and goals of the board were clearly established by all board members. Additionally, the board operating compact including roles, decision making, means of communication and rules of engagement were formed.

Through on-going regular meetings and extensive and structured discussions, a newly formed partnership was offered to other stake holders and Hmong community members to plant the first Hmong Genomic Board in Minnesota. In details, the board included a wide array of people with different levels of influence on the community while engaging individuals with different expertise in their respective fields. For example, the Hmong Genomics Board included Hmong and non-Hmong physicians, Hmong nurses, Hmong pharmacists and graduate student, Non-Hmong students, Hmong community members and university of Minnesota researcher investigators. This board represented the incarnation of the key principles of CBPR and the core group that jointly and collaboratively identified key health concerns within the Hmong community while expanding on the approach and design to address these concerns.

Results and Discussion:

After engaging in many interviews with key Hmong leaders and discussion amongst the board members, key lessons and guidelines were identified to be critical while working with the Hmong population. These lessons learned ranged from the basic understanding of the ethnographic nature of the Hmong to the more complex controversial issues associated with conducting a genetic-based research. For example, the Hmong language lacks terms that translate biomedical body physiology and anatomy. Therefore, explaining a disease state or organ malfunction would require the interpreter to use a lengthy paragraph to say what could be said with one word in the English language. This lack of sufficient terms in Hmong to correlate with the western medical terms can easily create major gaps in heath communication while widening the gaps of trusting western medicine. In addition, this language barrier may have other implications associated with the Hmong participation of clinical research in general and particularly genetic research. Therefore, creating consent forms and educational materials will require very special consideration given the limitations associated with the Hmong language. It was also decided that creating consent forms should be done using Hmong and English. The educational materials included more pictures to enhance communication rather than relying on words. For example, to explain heredity, layman language and cultural related examples (e.g. the man's seed and woman's egg) should be used to ensure full understanding of the topic presented to participants.

Interactions with the community prior to the onset of the study will allow potential participants to become more familiar with the researchers and enable researchers to gauge

whether their approach to the community is effective. These interactions can be mediated via multiple mechanisms. One of which is building a relationship with the community leaders, heads of the clans, healthcare professionals from the Hmong population. Other mechanisms could include Hmong radio and TV stations to increase awareness of the community and build mutual trust.

Study locations have been shown to play a major role in improving study participation and ensuring study participants returning to subsequent visits especially when the study involves multiple visits. Therefore, using clinical sites that are familiar to Hmong patients or using the same clinical sites from which they receive their regular medical care will substantially increase the likelihood of the participants to enroll into the study and complete the study if they needed to come back for multiple visits.

The nature and amount of the biological samples are important to Hmong participants. For example, relying on non-invasive biological samples such as saliva or mouth swab is more acceptable than using more invasive sample like blood. However, if the study purpose is hematological disorders for example, minimizing the amount of blood drawn will improve the participant's acceptance to be in the study. This is due to the belief system that some Hmong might have about having a finite blood volume, which makes them to believe that giving a large volume of blood can weaken their body and disrupt their reincarnation.

Future Research Opportunities and Cultural Perspectives

While the Hmong remain to be less represented in clinical or genetic-based research relative to other population groups, genetic-based investigations in the Hmong could not

only benefit the Hmong but may well enhance our understanding of the genetic basis of some common diseases in the public. Thus, our ***main objective*** is engaging the Hmong in clinical and genetic-based research to address some of their existing health disparities and advance precision medicine overall. Furthermore, the Hmong population may be ideal candidate for genetic exploration because the Hmong have a high cultural identify and a very low admixture rate outside their ethnic groups. These unique characteristics can it make easier to precisely address some of the key differences in prevalence of cardiovascular and non-cardiovascular diseases in the Hmong compared to non-Hmong independent of confounders in the pathogenesis of these diseases. This also leads us to postulate our ***central hypothesis*** that Hmong have a differential prevalence of disease-gene pair risk alleles and drug-gene pair response alleles that distinguish them from non-Hmong. Therefore, ***the long-term goals*** of our research are to reduce the health disparities present in the Minnesota Hmong and advance precision medicine by engaging unique minority populations in genetic-based research.

Concerning the most pressing health conditions affecting the Hmong, multiple disease states have been identified and reported to be substantially affecting a large percentage of the Hmong population. Some of these disease states included type 2 diabetes, hypertension, kidney diseases and gout. While all the disease states listed above are important and highly associated with high morbidity and mortality, gout is believed to be a unique case since it is strongly associated with all the other diseases as the "common denominator" as well as an independent risk factor to hypertension, obesity and chronic kidney disease.[18,23,54-57] In addition, anecdotal report on trends of Hmong's dietary

lifestyle may well suggest the increased risk of gout in the Hmong. Furthermore, exploration of the genetic basis of hyperuricemia and gout in the Hmong was recognized as step forward to address the needs of the Minnesota Hmong community. Such a recognition by the board members was the driver of ensuing work related to genetic investigations in the Hmong. Therefore, studies aimed towards greater understanding of the interactions between diet and genetics, which may lead to higher prevalence of gout in Hmong, is justified, supported by the community and medically warranted.

Chapter Summary

The Hmong is a unique racial group with high prevalence of gout and gout-related comorbidities. The Hmong population is underrepresented in clinical research especially that involves advancing genetic discoveries of disease risk. This gap of inclusion represents a missed opportunity and health disparity of knowledge. The dietary lifestyles of the Hmong parallel the enhanced risk of developing hyperuricemia and gout. The Hmong community identified gout as a priority health condition to collaborate on with academic researches. Studies that address the genetic architecture of the uric acid disposition in the Hmong, the effect of Hmong dietary lifestyles on hyperuricemia and gout, and possible implications of genetic basis for the response to drugs that are commonly used to manage gout are justified as means to improve the quality of care provided to Hmong patients with gout. Recognition of the role of including minorities, women, and sub-populations in genetic research by the NIH, underscores the importance to pursue research within the Hmong community to address their unique health disparities while advancing the ultimate goal of personalized medicine for the population at large.

Figure 1:1 Prevalence of gout by age categories and geographical locations
This figure suggests that gout seems to increase in a linear fashion with age.[10]

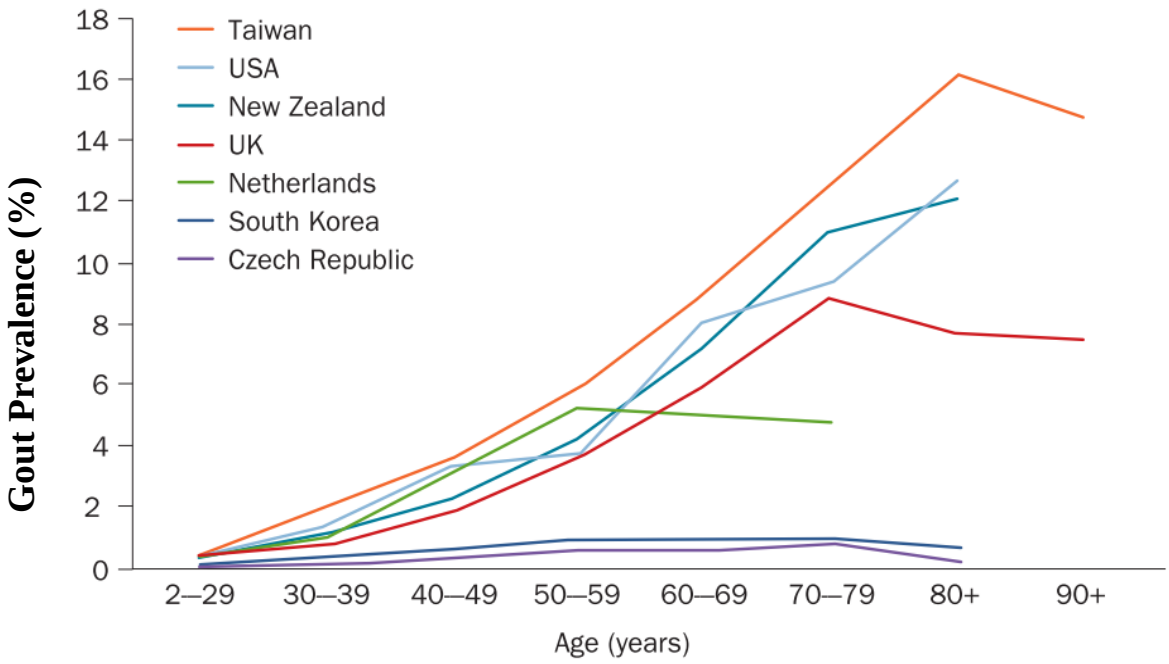


Figure 1:2 Prevalence of gout by sex and countries
Gout remains to be male dominated in countries with the highest prevalence of gout.[10]

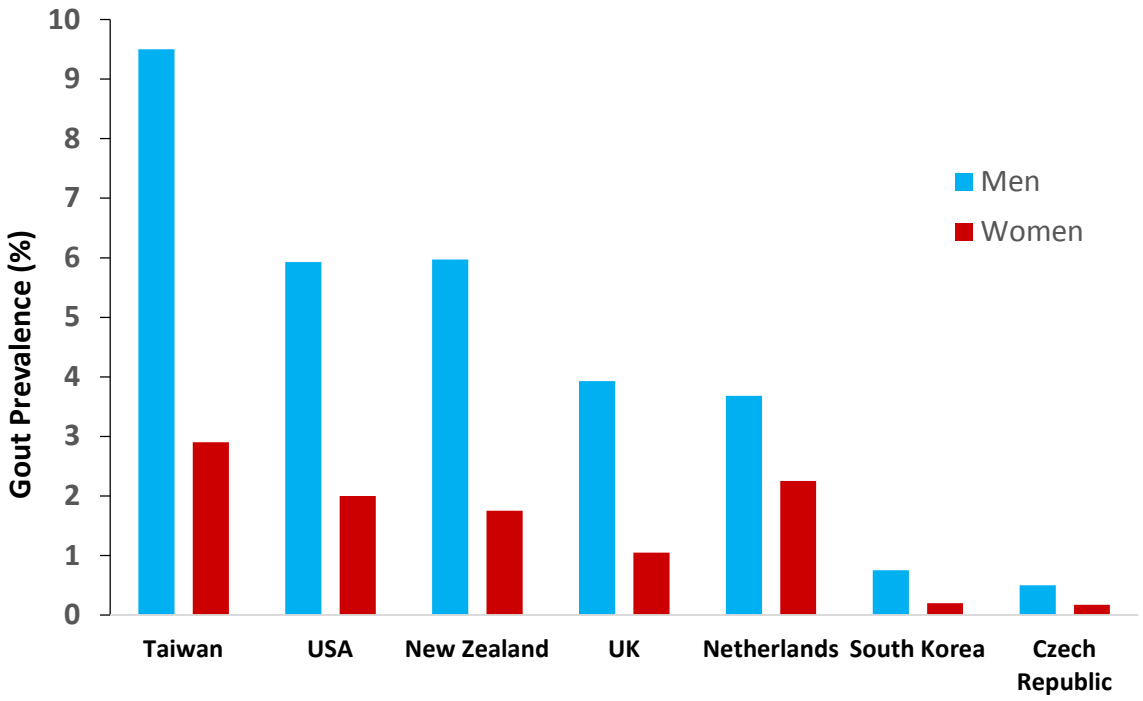
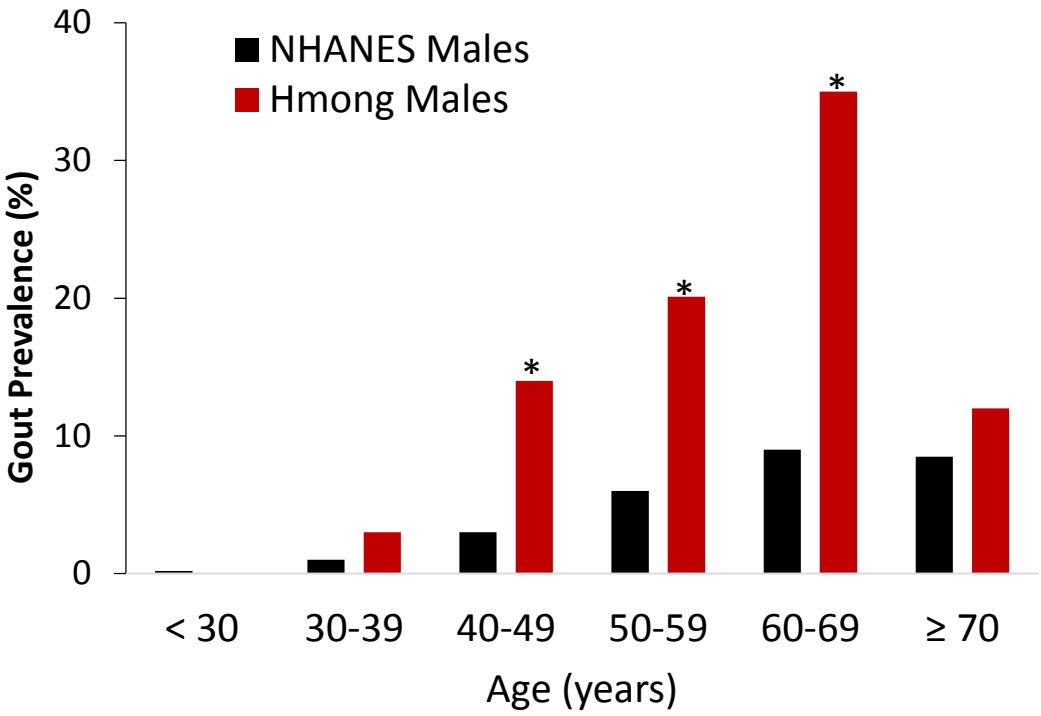
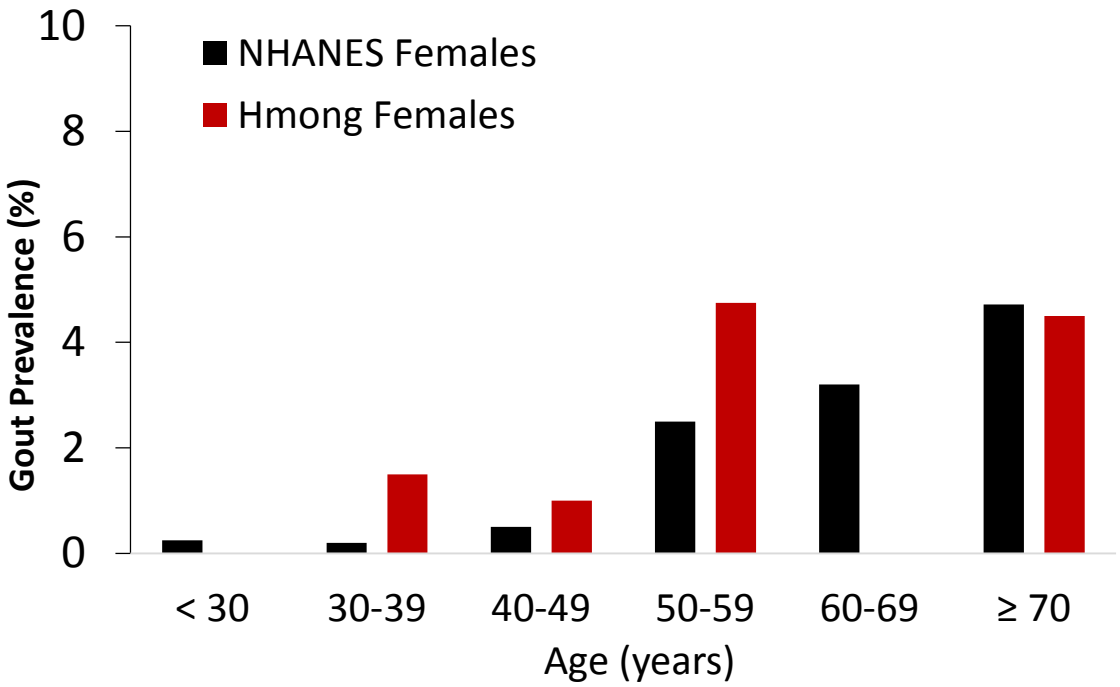


Figure 1:3 Prevalence of self-report gout in Hmong males vs. US sex and age-matched populations[17]



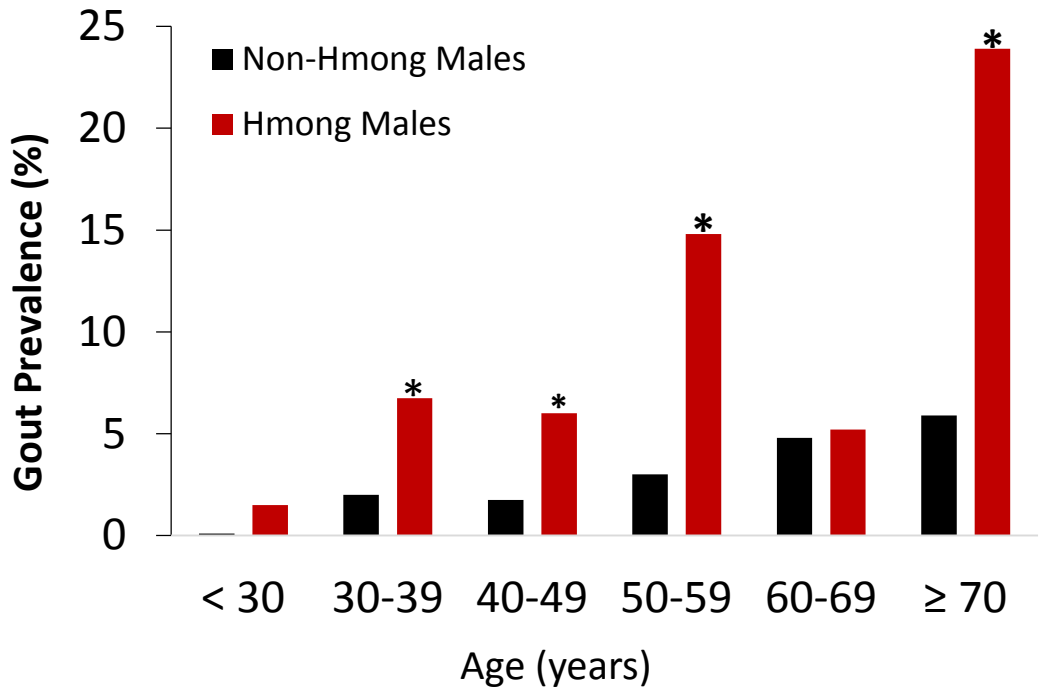
NHANES= National Health and Nutrition Examination Survey III (1988-1994)
*p<0.001

Figure 1:4 Prevalence of self-report gout in Hmong females vs. US sex and age-matched populations[17]



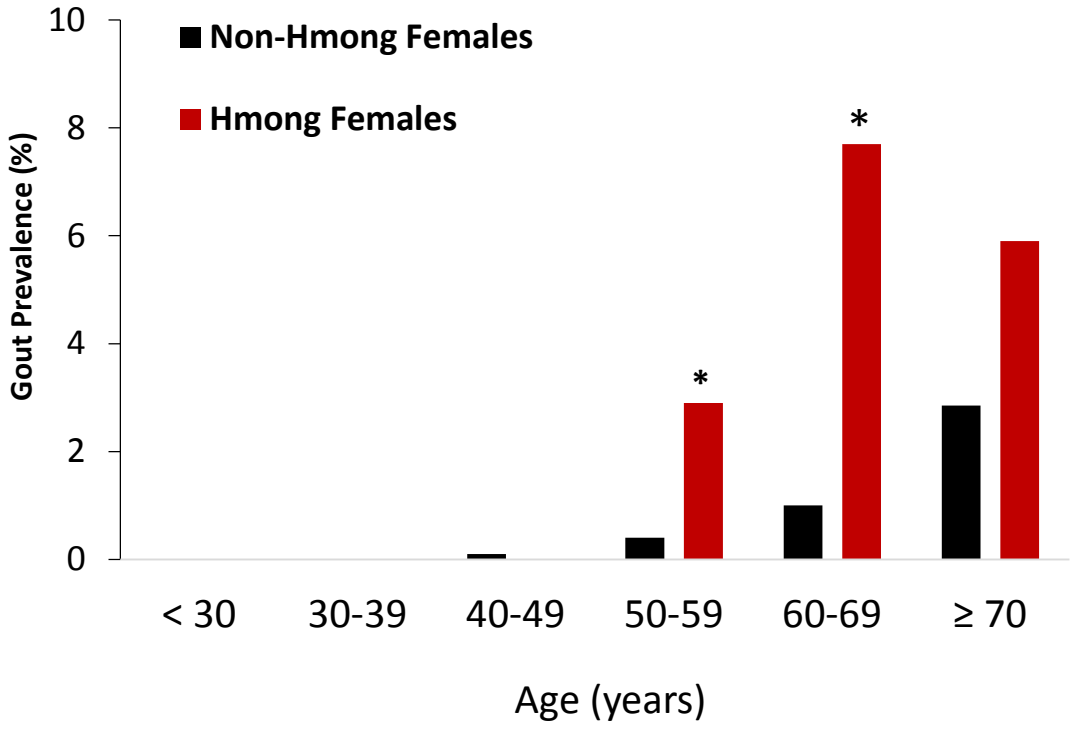
NHANES= National Health and Nutrition Examination Survey III (1988-1994)

Figure 1:5 Prevalence of physician-diagnosed gout in Hmong males vs. non-Hmong sex and age-matched populations[17]



*p<0.001

Figure 1:6 Prevalence of physician-diagnosed gout in Hmong females vs. non-Hmong sex and age-matched populations[17]



*p<0.001

Table 1:1 Scientific, cultural, and social factors to consider in tailoring informed consent for genomic research[49]
1. Study design (disease versus non-disease studies; selected genes versus whole genome)
2. Data and biological sample sharing requirements
3. Reporting study findings to participants
4. Cultural context of the study
5. Participant language and literacy
6. Participant knowledge of differences between research and clinical care
7. Potential for stigmatization of the study population
8. Inclusion of indigenous populations
9. Strength of economic, scientific and health infra-structures at study sites
10. Regulatory oversight

Table 1:2 Key Principles of Community-Based Participatory Research[53]
1. Recognize community as a unit of identity
2. Build on strength and resources within the community
3. Facilitate collaborative, equitable partnership in all research phases and involve an empowering and power-sharing process that attend to social inequities
4. Promote co-learning and capacity building
5. Integrate and achieve balance between research and action of mutual benefit of all partners
6. Emphasize public health problems of local relevance and ecological perspectives that recognize and attend the multiple determinants of health and disease
7. Involve systems development through cyclical and iterative process
8. Disseminate findings to all partners and involve all partners in the dissemination process

Chapter 2 Chapter II

Review Article

Uric Acid, Hyperuricemia and Gout Contemporary Overview

Chapter Overview

This chapter gives a comprehensive overview of the history, economic burden, pathophysiology, and pharmacotherapy of gout. Through this chapter, the reader should be able to appreciate that the optimal management of gout does not only reduce the risk of recurring gout flares, but can greatly reduce the burden of the comorbidities associated with gout.

Gout: The Disease of Distinction

Gout is an inflammatory arthritic condition caused by the deposition of monosodium urate crystals within the distal joints. The term gout is derived from the Latin word *gutta*- to drop. This term was coined by a Vielehardouin in the 13th century based on the humor theory that a fluid ran down the body into the legs.[58,59] Gout is relatively one of the oldest known diseases in the history of medicine. The first gout-like cases have been recognized and documented by the Ancient Egyptians in 2640 BC. In the 5th century BC, gout was recognized by Hippocrates and named as the "*unwalkable disease*" reflecting the reduced mobility and excruciating pain associated with gout. Hippocrates' work to further understand the relationship between gout and other modifiable or non-modifiable risk factors has provided the foundation for key insights into risk factors for and management of gout. In fact, Hippocrates's principles on the pathophysiology of gout some 2500 years ago, such as age and sex hormones, still carry clinical relevance today (Table 2.1).[58,59]

Throughout history, acute gout occurring due to monosodium urate crystallization in the metatarsophalangeal joint (aka podagra) has been associated with meat rich food, excessive alcohol consumption, and certain lifestyles that could be afforded by a select

upper class of the society. Consequently, gout was referred to as "*the disease of the kings*".[58,59] Therefore, gout as a disease conferred a high social status and implied distinction. Moreover, having gout carried a special message because while it inflicted an excruciating pain, it rarely killed; thus, it was perceived as a death prophylactic disease. In addition, gout was historically known as "*the old male disease*" due to the notion that young men do not develop gout unless they indulge in sexual activities. On the contrary, the number of women affected by gout is far less than men but increases especially after menopause. With a history spanning more than 2500 years ago, gout did not just afflict the quality of life of its sufferers, but has also played a major role in arts and politics.[60] While being influenced by Hippocrates' views on gout, Michael Psellos (1016-1078 AD), a famous Byzantine monk, philosopher, writer and politician wrote a 15-lines poem titled "*An Excellent Medical Work in the Iambic Manner*" to describe the nature of acute and chronic gout. This poem could be considered as the first extensive documentation of the clinical signs and pathogenesis of not just the monoarticular form of gout (podagra) but also the polyarticular gouty arthritis.[61]

On one hand, many politicians and key decision makers have suffered gout and experienced major gout attacks on days that resulted in their absence and precluded them from voting on major and historical decisions. On the other hand, some also believe that having gout has also unified a group of politicians on some key decisions that shaped our modern history. For example, it is believed that gout affliction has crystalized the relationship between Franklin, Jefferson, and Comte de Vergennes and has played a role in the origins and outcomes of the American Revolution as well as the Declaration of

Independence.[59]

Epidemiology of Hyperuricemia and Gout

The global prevalence of gout and its strongest predictor, hyperuricemia, have been on the rise in the past few decades. Consistently, data from major healthcare systems and the Health and Nutrition Survey(NHANES) in the U.S. have shown a rise in prevalence of hyperuricemia and gout(Table 2.2).[11] For instance, descriptive data analysis of prevalence of gout and hyperuricemia over 10 years (1990-1999) in older adults in a managed care population demonstrated that ongoing increased prevalence of hyperuricemia and gout. While gender and age are important determinants in the development of gout, the overall prevalence of clinically significant hyperuricemia and/or gout, increased from 2.9 per 1000 enrollees in 1990 to 5.2 per 1000 enrollees in 1999. In addition, the results showed that gout remains a disease of men (3:1, men to women) and the elderly noting that those >75years had the highest prevalence of gout per 1000 enrollees per year (Figure 2.1).[62] Furthermore, using data from 5,707 participants in NHANES conducted in 2007 and 2008, the mean serum urate levels was 6.14mg/dL among men and 4.87mg/dL among women.

The overall prevalence of hyperuricemia was 21.4 % in the US.[11,18] Specifically, the prevalence of hyperuricemia is 21.2% among men and 21.6% among women. This estimated prevalence considers the gender difference in the definition of hyperuricemia (>5.7 mg/dL vs. > 6.8 mg/dL) since women tend to have lower UA levels compared to men. Although prevalence of hyperuricemia is nearly similar across genders, data from the NHANES 2007-2008 shows that the prevalence of gout varies between men (6%) and women (2%). This differential classification of hyperuricemia and prevalence of gout

between genders may have a variety of sources; one of which reflects the effect of circulating estrogen levels. Estrogen is known to have a uricosuric effect.[63,64] However, the impact of gender on the prevalence of hyperuricemia or gout becomes less influential as women reach the postmenopausal age where circulating estrogen levels decline. The effect of age, however, might have a more significant impact on the development of hyperuricemia and gout. It is expected that as age increases, the risks of developing hyperuricemia and gout also increase due to declining kidney function, risk of dehydration, increased use of drugs, and increased prevalence of other comorbidities. Thus, the combination of declining in estrogen levels and the complications of aging suggest women could be at higher risk for developing gout at later stages in life than men. It is also noted that prevalence of gout substantially differs across geographical regions of the world. While Greece presents with the highest prevalence of gout in Europe (4.75%), other countries like the Philippines, Jamaica, Iran, and African countries have reported prevalence of < 1% or it could be minimally reported in other parts of the world such as the South Korea (0.4%).[10] While the effects of age and sex on the incidence of hyperuricemia and gout are well established, this geographical differential prevalence of gout further supports the role of environment and dietary habits as well as other lifestyles which could modulate the risk of developing gout.

There are multiple limitations associated with the methods used to assess the prevalence or incidence of gout. Some of the most encountered shortcomings are relying on participants' self-report, which is confounded by recall bias, sampling limitations, misdiagnosis or case-definition of gout, and the timing of conducting the study. Owing to

these limitations and variation of methodologies used to estimate the prevalence and incidence of gout, the need for replication and longitudinal studies are warranted. This is to accurately quantify the prevalence and incidence of gout, which is a debilitating and costly disease to its sufferers and society at large.

Economic Impact of Managing Patients with Gout

Gout is associated with a substantial economic and humanistic burden to the healthcare system and its sufferers. The burden of gout on the society includes direct and indirect costs. For example, missed days of work, loss of productivity, urgent care visits, and hospitalization all can add up a substantial economic stress on the induvial as well as the society. The estimated direct cost (2004 values) to manage a patient with gout is $6871 per patient/year in contrast to $3705 to manage a patient without gout.[65] Furthermore, using data from the 2005-2011 Medical Expenditure Panel Survey, it is estimated that mean per-person all-cause medical expenditures were more than 2.5-fold higher for adults with gout compared to the entire adult population without gout, mostly driven by hospitalization and inpatient stay. In addition, the total annual national medical expenditure attributable to gout was $7.7 billion, accounting for almost one of four dollars spent for medical care of the US adults with gout.[66] A systematic review has also assessed the annual incremental direct cost of gout in those experiencing regular acute flares or have tophi present at 2004 and 2005 compared to 2008.[67] The estimated cost ranged from $3165 to $5515 in 2004 to 2005, respectively. Using the 2008 data, however, the incremental direct cost of gout climbed to $10,222 and up to $21,467 in patients experiencing six or more gout flares per year.[67]

Although there is a lack of a universal instrument to assess the health-related quality of life in individuals with gout, the three mostly commonly used tools are the Health Assessment Questionnaire Index (HRQOL), Short Form 36 (SF-36), and its modified form known as the Short Form 6-dimensions (SF-6D). Using these instruments, the humanistic burden of gout can be quantified using a numerical scale a with maximum score of 1. These scores are positively correlated with the health-related quality of life and are negatively associated with acute gout flares. For instance, a systematic review has showed that patients who experienced $\geq$3 acute flares/year had SF-6D utility score 0.53 compared with 0.73 in gout patients who were asymptomatic or experienced mild symptoms.

While the listed above quality of life assessment tools are inherently different in their scopes and discriminative powers to assess a subjective reporting of quality of life in patients with gout, the SF-6D scores derived from the SF-36 may have a higher discriminative power in a population health survey.[68] However, the ability to determine which factors that predict changes in the health-related quality of life to identify those at risk to deteriorate and be targeted for treatment will constitute the most optimal method of assessment.

Pathogenesis of Hyperuricemia and Gout

Serum Uric Acid (SUA) is the end-product of purine metabolism. Sources of purines can include both endogenous such as the breakdown of nucleic acids, or exogenous from dietary sources. Purines are generally converted to hypoxanthine, xanthine and ultimately uric acid via the xanthine oxidase (XO) enzyme (Figure 2.2). Based on an average purine content diet, approximately 5-6 mmol (~800-1000mg) of uric acid is produced daily.[69] Of

this amount, 3-4 mmol (~500-600mg) of urate is produced endogenously while the remaining 1-2 mmol (~200-300 mg) is produced from dietary sources of purines.[69] Of the amount produced daily, approximately 70% of the uric acid is excreted by the kidney while the remainder is eliminated via the gastrointestinal tract, where it is degraded by bacterial uricase.[70]

The role of uricase-producing bacteria in the intestine imposes the question of whether changes in gut microbiome may have an influence on the risk of developing gout. A study of gout patient cases (n=35) compared to healthy individuals (n=33) has identified that the intestinal microbiota profiles are distinct in both the organismal and functional structures. In fact, the intestinal microbiota of patients with gout are comparable to those of type-2 diabetes and metabolic syndrome. Furthermore, the reference microbial gene catalogue for gout cases revealed disorders associated with purine metabolism and butyric acid biosynthesis both of which to be the basis for developing gout. [71]

Elevated SUA is known as hyperuricemia and the strongest predictor of developing gout. As mentioned above, hyperuricemia is a function of dietary intake of purine sources, endogenous cell turnover and renal as well as extra renal eliminations of UA. In theory, changes in any of these factors affecting UA disposition, may account for hyperuricemia. In fact, defective renal elimination of UA accounts for approximately 80-90 % of gout cases knowns as UA underexcreters due to decreased urinary UA secretion. The remaining cases known as overproducers or because of renal UA overload or combined mechanisms (underexcretion of overproduction).[70,72] Endogenously, sources of UA are cell turnover or de novo synthesis of purines. Therefore, hyperuricemia can be divided into two major

types: primary hyperuricemia and secondary hyperuricemia. The causes of primary hyperuricemia can be further divided into two main categories. The first category which results from UA overproduction which accounts for 10% of the population with gout.[73] While the over-production of UA can be an inherited genetic disorder as in the case of Lesch Nyhan Syndrome or a result of Tumor Lysis Syndrome, high intake of purine sources can also result in high SUA levels and the development of hyperuricemia.[69]

The second category of primary cause of hyperuricemia is attributed to the underexcretion of UA which accounts for 80-90% of patients with gout.[70] Uric acid is extensively (90%) reabsorbed from the kidney via the Four Component Theory (Figure 2.6),[74] which allows a small fraction of UA to be excreted. In fact, underexcretion of UA tends to be the dominating mechanism of developing hyperuricemia, which is supported by the observation that most of the patients with declining or poor kidney function tend to have higher SUA levels than those with normal kidney function. On the other hand, kidney function may not always explain the underexcretion associated with hyperuricemia as a substantial number of individuals with normal kidney function but still have higher baseline than the normal range for SUA levels. The etiology of such discordance between kidney function and SUA levels in some individuals has been attributed to genetic variants within select uric acid transporters genes mainly *ABCG2, SLC17A1 and SLC22A12* in the kidney, which can increase SUA levels even in healthy individuals. The interaction of these genetic variants can render some to more efficient at reabsorbing uric acid while making others less efficient at secreting UA.

Genetics of Hyperuricemia and Gout

Hyperuricemia and gout are highly heritable with estimates of up to 45[75] and 65%[76], respectively. The recent advances in genetic tools applied to large populations such as genome-wide association studies (GWAS), have helped to elucidate major genetic variants in UA disposition pathway, which may predict hyperuricemia or gout. The genes which have been identified to be associated with hyperuricemia mainly involve genes affecting uric acid excretion (*ABCG2*, *SLC17A1*), uric acid reabsorption (*SLC22A12*, *SLC2A9*, and *SLC22A11*) and a lipid metabolizing gene (*GCKR*).[77] In addition, there are other genes responsible for supporting the pathway of UA disposition such as the scaffolding protein (*PDZK1*) which also play a role in affecting baseline SUA. The differential prevalence of hyperuricemia and gout coincides with race. For example, some racial groups such as African-Americans, Japanese[12], and Hmong[17] tend to have a higher prevalence of gout compared to Caucasian[78] and European.[76] This racial differential prevalence of hyperuricemia or gout also parallels the prevalence of genetic variants mainly single nucleotide polymorphisms (SNPs) consistent with the SNPs identified by GWAS.[79-82] These genetic findings may play a role in the explanation for why some the populations are known to have the highest documented prevalence of gout such as the Maori and Aboriginal populations.[9,10]

Although these genetic variants are found to be differentially prevalent across racial groups making certain populations to be at higher risk for the aforementioned diseases, at least one group studying several populations found that the magnitude and direction of impact these genetic variants were consistent across most of the studied populations.[81] The

etiology of gout is a heterogeneous process involving genetic and non-genetic factors. While key genetic variants associated with baseline SUA or gout have been identified, recent candidate genes studies and GWAS have suggested some genetic variants to be associated with the gout subtypes: overproduction, underexcretion or combined in Japanese patients with gout.[83] Specifically, genetic variants were found in *SLC16A9* and *SLC22A11*, which may predict the overproduction and underexcretion of uric acid phenotype, respectively.[84,85] This interplay between genetic polymorphisms within transporters and the therapeutic drugs that interact with these transporters highlight the potential value of genetic knowledge to guide drug therapy. For example, these genetic polymorphisms, mainly SNPs, could also influence the response to urate lowering therapy, mainly allopurinol[86] or possible side effects from co-administered drugs in patients with hyperuricemia or gout. A comprehensive depiction of the urate transportome as it relates to the different transporter genes associated with handling of uric acid in the kidney is presented in Figure 2.4.

Risk Factors for Hyperuricemia

There are modifiable and non-modifiable risk factors for developing hyperuricemia. The non-modifiable risk factors include age, sex, genetics, and race. As the person ages, kidney function typically declines and the ability to excrete UA declines. In addition, age is a key predictor of the development of chronic diseases for which the treatments often involve drug therapies which can interfere with UA disposition. With respect to gender, gout is more common amongst men compared to women. However, the prevalence of hyperuricemia is similar between both genders. This is due to the differential definition

cut-off for hyperuricemia between genders which is higher for men than women ($\geq$ 6.8 mg/dL vs. $\geq$ 5.7 mg/dL). This differential definition is due to the uricosuric effect of estrogen, which in part explains why women have a lower incidence of gout. However, postmenopausal women tend to have a similar risk for developing gout compared to men. In men, however, androgens may increase systolic blood pressure by increasing the sodium and water retention through the activation of the renin-angiotensin aldosterone system (RAAS)[87]. Moreover, elevated SUA levels have been reported in patients receiving testosterone replacement therapy.[88] Evidently, hormonal levels modulate SUA through different mechanisms across genders. Although hypothesis generating, one may theorize that the changes in androgen levels with aging can modulate the risk of developing gout. However, changes in renal function may overwhelm the impact of hormonal effects on the risk of gout with aging.

Dietary and social lifestyles are also important factors in the development of gout and hyperuricemia. It is well documented that higher intake of purine sources, especially red meat and seafood, can worsen existing gout symptoms or unmask existing risk for developing gout. In addition, higher intake of high fructose corn syrup sources such as sweetened soft drinks can result in new onsets of acute gout attacks.[32,89] Furthermore, sugar-sweetened soft drinks especially those with fructose can significantly increase the relative risk for gout with 1.85 [95%CI, 1.08 to 3.16].[90]

Fructose consumption has dramatically increased over the past a few decades nationally and worldwide.[15] This observation has been cited as a contributing factor responsible for some of the increase in the prevalence of hyperuricemia and gout in

countries where fructose is commercially used as a sweetener.[15] The mechanism by which fructose increases SUA is not clearly understood; however, it has been proposed that fructose gets reabsorbed from the kidney via the GLUT-9 transporter[91] (Figure 2.4) allowing high serum fructose levels, which are metabolized by fructose 1-phosphate via the rapid activity of fructokinase that takes place in the liver. This conversion results in a sharp depletion of ATP subsequently increasing uric acid levels (Figure 2.5). The depletion of ATP pool further stimulates the *de novo* synthesis of purine nucleotides pathway causing more UA production.[89] In addition, a substantial amount of ingested fructose gets converted into lactate, which competes with uric acid excretion causing an increase in SUA. The effect of lactic acid on uric acid is supported by the observation that fructose-induced hyperuricemia is associated with decreased renal uric acid excretion in human.[92-94] Collectively, these mechanisms are by which consumption of high fructose corn syrup can increase the risk of developing gout or the development of gout attacks.[32]

Select forms of alcohol have also been implicated in the development of gout and/or exacerbation of gout attacks. This observation is mainly due to the varying levels of purine contents which are the precursors of UA. Furthermore, the effect of alcohol on UA production results from the conversion of ethanol into Acetyl-CoA, which leads to degradation of purine trinucleotides increasing levels of UA precursors. Like fructose, the formation of lactic acid from ethanol metabolism further decreases UA excretion leading to higher SUA. It is also important to note that some alcoholic drinks could have a higher effect on uric acid production owing to the high purine contents and percent of alcohol involved. For example, beer tends to have the greatest effect on uric acid, while wine

shows the lowest risk for worsening gout symptoms or new onsets of gout attacks.[16]

Uric acid is a weak acid with a pKa of 5.8 and predominantly found in the ionized form, urate. Since urine pH is critical for determining the amount of uric acid that is reabsorbed from the proximal convoluted tubule (PCT), we can postulate that chronic exposure to pH modifiers can have a substantive effect on the amount of ionized form of urate in the PCT. Thus, dietary or non-dietary sources that can acidify the urine may enhance the reabsorption of uric acid causing the buildup of uric acid in the kidney resulting in an acute uric acid nephropathy and possible uric acid kidney stones.[38,39]

Secondary Hyperuricemia

Drug-Induced Hyperuricemia

Diuretics are some of the most commonly used drugs in the management of chronic diseases such as hypertension, fluid overload and heart failure. They are also associated with elevated SUA and significantly increase the risk of worsening gout symptoms and new onsets of gout flares.[95] In order to assess the risk of developing gout with the use of diuretics, an analysis of large population-based cohort from the Atherosclerosis Risk in Communities study also known as the ARIC study, identified that the use of any diuretics: thiazide or loop was significantly associated with new incidence of gout compared to no use of diuretics (HR 1.48 [95%CI, 1.11 to 1.98]). Although not significantly different, the loop diuretics had a higher risk (HR 2.31 [95% CI, 1.36 to 3.91]) than thiazide diuretics (HR 1.44 [95% CI, 1.00 to 2.10]) with incident gout (Table 2.3).[96] Diuretics are proposed to affect uric acid by direct and indirect mechanisms. The direct effect is marked by decreased uric acid excretion through the kidney by simply competing for excretion at the basolateral and apical sides. The indirect effect is mediated via the enhanced uric acid

reabsorption by the exchange of thiazide excretion at the Uric Acid Transporter 1 (URAT1) and Organic Anion Transporter (OAT) [Figure 2.5]. Another key uric acid transporter is known as Multi Drug Resistance Protein 4 (MRP4), which is expressed on the apical side of the proximal tubule and is inhibited by certain thiazides and loop diuretics.[97] Therefore, the inhibitory effect on MRP4 may explain the side effects of high uric acid associated with high doses of diuretics.[97]

Although loop diuretics could modulate the risk for hyperuricemia or gout, they can also modulate the pharmacokinetics of urate-lowering drugs such as allopurinol. The co-administration of loop diuretic such as furosemide with allopurinol is common in clinical practice especially in patients with gout and heart failure. Notably, furosemide does not only increase uric acid levels in individuals receiving allopurinol, but also the levels of oxipurinol. However, the increased levels of oxipurinol in patients receiving the combination was not associated with greater reduction of SUA.[98,99] This interaction highlights the interplay of transporters associated with uric acid and oxipurinol disposition as well as the levels of XO expression in the presence of furosemide.[100]

Aspirin is a cyclooxygenase-2 inhibitor and at the doses of 65-325mg/day, is widely used for primary prevention of stroke and cardiovascular diseases as well as secondary prevention of cardiovascular events in select patient groups. Aspirin has a biphasic effect on SUA levels. Specifically, at the lower dose (< 2.5gm/day), aspirin can increase SUA levels by competing with uric acid renal tubular excretion transporters mainly: OAT1, OAT3, and GLUT-9. At the higher doses, aspirin blocks the renal tubular excretion and renal tubular reabsorption mainly URAT1; however, the net effect of aspirin at the higher

doses is uricosuria.[101] This phenomenon is explained by the fact that the renal tubular excretion plays a lesser role compared to the renal tubular reabsorption, which further explains the net uricosuric effect of aspirin at the higher doses.[102] To illustrate, a small randomized clinical trial tested the effect of 60mg (n=18) vs. 300mg (n=14) aspirin on the renal handling of uric acid and renal function over the course of two weeks. The study concluded that while either dose did not affect SUA or serum creatinine levels, both doses significantly reduced the fractional excretion of uric acid by the second week[103]. Although the study duration is relatively short to show a substantive effect on SUA, the significantly reduced FEUA% is consistent with the effect of aspirin on UA especially at the lower doses.

Nicotinic Acid (Niacin or vitamin B3) is a supplement that is associated with increased SUA. Niacin is also used to manage patients with dyslipidemia to increase high-density lipoprotein (HDL) and lower low-density lipoprotein (LDL) and triglycerides. Besides the well-documented side effects of flushing and GI disturbance, niacin can increase uric acid at the therapeutic doses ($\geq$1500mg/day) used to treat patients with dyslipidemia. The proposed mechanism of nicotinic acid associated hyperuricemia involves two key transporters. These transporters are the URAT1 (*SLC22A12*) and OAT10 (*SLC22A13*), which facilitate nicotinic acid excretion at the expense of uric acid reabsorption.[97,104]

Testosterone has been also associated with increased SUA. This, in part, explains why males tend to have a higher level of SUA compared to females. The mechanism by which testosterone increases uric acid was investigated in orchietomized mice model in

which testosterone was replaced exogenously. It was noted that testosterone replacement was associated with increased levels of mRNA and protein of the URAT1 and Sodium-coupled Monocarboxylate Transporter (SMCT1). This further suggests a plausible mechanism for the high uric acid associated with testosterone replacement therapy via the enhanced uric acid reabsorption mechanism.[105,106] In addition, it is believed that testosterone in part also increases muscle mass which itself a major source of purines explaining the rise in SUA with testosterone replacement therapy.[88]

Cyclosporine and tacrolimus are widely used immunosuppressant drugs and commonly used in solid organ transplant patients. Hyperuricemia has been strongly associated with their use although the exact mechanism is not clearly understood.[107] However, it is presumed that both drugs may be interacting with OAT10 (*SLC22A13*) transporter causing SUA levels to rise. On one hand, amlodipine was prospectively shown in one study to have significantly reduced uric acid levels in cyclosporine-induced hyperuricemia patients who were hypertensive renal transplant recipients.[108] On the other hand, amlodipine has shown to increase cyclosporine trough levels with up to 40% .[108] Although it is known that amlodipine may reduce the metabolism of cyclosporine levels by inhibiting CYP3A4, this study did not document any significant change in cyclosporine dose as a consequence of this interaction. Additionally, there was no change in serum creatinine, blood urea nitrogen, or mean arterial pressure values before and after amlodipine therapy.[109] While the interplay between amlodipine and cyclosporine would suggest cytochrome P450 interaction, mainly CYP3A4, the interplay between cyclosporine, amlodipine, and uric acid would suggest a transporter-mediated interaction.

In addition, one might think that although amlodipine can increase cyclosporine levels, it is believed the changes in the eGFR because of amlodipine could counteract the effect of vasoconstriction-induced cyclosporine hence enhancing uric acid clearance.[108]

While gout and hypertension have been linked, the effect of anti-hypertensive drugs have been also known to modulate the risk of gout. A nested case-control study design from the health improvement network database from the United Kingdom general practice conducted from 2000 and 2007, was used to assess the relative risk of incident gout associated with antihypertensive drugs.[95] After adjusting for age, sex, body mass index, visits to the general practitioner, alcohol use, and pertinent drugs and co-morbidities, the relative risk of incident gout associated with the use of different antihypertensive drugs in patients with hypertension (n= 29,138) was different by drug class (Table 2.3). Notably, the multivariate analysis of relative risks of gout showed that there were both duration and dose associations with the effect those drugs have on the risk of gout. For example, the relative risk for calcium channel blockers was 1.09 for use less than one year, 0.89 for use of 1-1.9 years, and 0.77 for more than two years. In total, 12,858 (51.9%) patients with gout had a recorded diagnosis of hypertension before the diagnosis of gout. After adjusting for age, sex, calendar year, and visits to a general practitioner, the relative risk of incident gout with hypertension was 1.99 compared to those without hypertension.[95] This suggests that certain antihypertensive drugs can increase the risk of gout when used long-term such as beta-blockers and diuretics while others can reduce SUA such as losartan and amlodipine [Table 2.3].

Theophylline is a methylxanthine bronchodilator that is used in patients with chronic

obstructive pulmonary disorder. Although the metabolism of theophylline involves multiple cytochromes P450 enzymes, xanthine oxidase plays a major role in the formation of the active and inactive metabolites of theophylline. While the use of theophylline remains limited due to its narrow therapeutic window, significantly elevated SUA levels were documented in asthmatic patients using theophylline compared to controls.[110,111] Although the exact mechanism by which theophylline can raise SUA levels remains inconclusive, it is presumed that theophylline raises uric acid production by enhancing purine catabolism by slightly inhibiting the activity of hypoxanthine/guanine phosphoribosyl transferase, which is a critical enzyme in the salvage pathway for purine synthesis.[112,113]

Disease-Induced Hyperuricemia

Acute exposure to higher levels of SUA has been clearly linked to the development of acute uric acid nephropathy, urolithiasis, formation of uric acid kidney stone and gout. For example, enhanced uric acid production due to rapid cell turnover, as in the case of tumor lysis syndrome or cell overgrowth as in the case of malignancies and leukemia, are strongly correlated with enhanced risk for acute kidney nephropathy. Chronic primary kidney disease, however, can be linked to chronic hyperuricemia and it has been proposed that the increasing prevalence of end-stage-kidney disease may be a result of the rising prevalence of gout.[57] Other more complex diseases such as metabolic syndrome tend to be highly associated with hyperuricemia.[20] Although it is controversial whether hyperuricemia is the cause of metabolic syndrome versus hyperuricemia is the culmination of metabolic syndrome,[114] it is well documented that metabolic syndrome is more prevalent in patients with gout versus those without gout. According to the NHANES III

(1988-1994), there is a significantly graded associated prevalence of metabolic syndrome defined by the NCEP/ATP with hyperuricemia with estimated prevalence of 18.9% for SUA <6mg/dL versus 70.7% for SUA ≥ 10mg/dL.[19] These observations of the association of hyperuricemia and metabolic syndrome, kidney disease and cardiovascular diseases, elevate the importance of managing hyperuricemia in the context of comprehensively addressing cardiovascular disease risks. Summary of primary and secondary causes of hyperuricemia subtypes are listed in Table 2.4.

Hyperuricemia Secondary to Renal Insufficiency

The defective renal elimination accounts for approximately 80-90 % of hyperuricemia cases (underexcreters) while the remaining cases results from overproduction or combined mechanisms.[69] Hyperuricemia and renal insufficiency are common clinical presentations that trigger a debate on the nature of the relationship between these two conditions. Although urate levels may not rise in patients with mild to moderate kidney function, urate levels tend to be markedly elevated in patients with creatinine clearance below 30mL/min. Conversely, FEUA% could rise exponentially when creatinine clearance falls below 30mL/min (see equation below) in patients with hyperuricemia secondary to renal insufficiency.[69] This high FEUA% in this patient population may confound their hyperuricemia or gout assessment based on being underexcreters, overproducers, or both (Tables 2.5-6).

$$FEUA = \frac{Uric\ Acid\ mg/dL(urine) * Creatinine\ mg/dL(Serum)}{Uric\ Acid\ \ mg/dL(Serum) * Creatinine\ mg/dL\ (Urine)} \times 100$$

Uric Acid Reference Levels Confounders

The reference range of serum urate is based on the levels at which there is an

increased risk of developing gout. As discussed earlier, levels of SUA can be affected by select drugs or changes in the kidney function. However, other variables can greatly modulate serum urate outside the normal reference levels [Table 2.7]. These variables include age, sex, race, genetics pregnancy and diet. Therefore, assessment of these variables should be incorporated in the patient's initial evaluation for hyperuricemia or gout.

Age and sex

In males, the annual incidence rates for the first gout episode exponentially increase with SUA at or greater than 0.42mmol/L (~ 7.1mg/dL), which coincides with the saturation value of urate in plasma at 37°C.[69] On average, women tend to have about 0.06mmol/L (1mg/dL) urate levels lower compared to men, which is attributed to the uricosuric effect of estrogen. On the contrary, women tend to be affected by rheumatic diseases far greater than men. However, gouty arthritis seems to affect a relatively a small fraction of women (3-5%) compared to men (5-12%) with the overwhelming majority at the postmenopausal age.[115]

Race and genetics

The prevalence of asymptomatic hyperuricemia among Polynesian women (Maori, Cook Islanders, Samoans and Tongans) is doubled compared to Caucasians.[9] This high prevalence of hyperuricemia is predominantly caused by the reduced FEUA% and is not associated with renal disease. The urate excretion rate is reported to be even lowers in Polynesian males.[69] These findings further support the hypothesis that the indigenous pacific races share a similar genetic defect in renal handling of urate rather than a renal dysfunction causing hyperuricemia. The exact mechanism that genetics can affect the risk

of hyperuricemia or gout is discussed in more depth in the next chapter.

Pregnancy

Urate levels initially fall by up to 25 % in the first trimester but rise to values of 20% higher than non-pregnant state. However, the urate levels are markedly elevated in pre-eclampsia events.[69,116]

Diet

The recommended reference interval of 24-hours urine uric acid collection in patients on an average or normal purine content diet is 1.5-4.5 mmol/day (250-750mg/day). In general, uric acid excretion can be either decreased or increased in response to a variety of pharmacologic agents. Nonetheless, the urinary uric acid excretion rate is elevated in a significant proportion of patients with uric acid stones and in those with states of uric acid overproduction such as in leukemia and polycythemia and after intake of food rich in nucleoproteins.[69]

Risk Factors for Hypouricemia

There are select and well documented lifestyles that can decrease SUA and reduce the risk of gout. While weight loss and regular physical activity can decrease SUA in the long-term, some dietary and food sources have been also associated with lowering SUA.[117] For instance, adequate hydration is very important to promote uric acid filtration and subsequently excretion. This lifestyle is highly recommended in individuals who tend to have a higher risk for forming kidney stones. As mentioned previously, the acidification of urine can increase UA reabsorption; in contrast, the alkalization of urine can greatly enhance UA excretion. In fact, one of the treatment modalities of managing gout and uric acid kidney stones is the use of potassium citrate, which increases the pH of the urine

stimulating stone dissolution through increasing uric acid excretion. Dietary sources that could increase urine pH are low-fat dairy products e.g. skimmed milk.[38]

While high-purine contents food can adversely affect gout, or trigger a gout attack, cherries have been shown and reported to ameliorate gout attacks and may reduce SUA. An internet-based observational case-crossover study has assessed the effect of cherry consumption in 633 patients with gout during 2-day hazard period (prior to gout attack) compared to 2-day control period.[118] The study identified that consumption of any cherries was associated with a 35% lower risk of gout attack compared with no consumption. Additionally, the risk of gout attack was further lower with increased consumption of cherries with up to 3 servings over 2-days. Notably, the study identified that the exposure to the combination of allopurinol and cherries had 75% lower risk of gout attacks compared to without either exposure.

Although the study by Zhang *et al* [118] did not assess the effect of cherry consumption on SUA levels, Jacob *et al* [119] investigated the effect of consuming two servings of cherries on plasma urate in 10 healthy women. The consumption of cherries was significantly ($p<0.05$) associated with reduced plasma urate at 5 hours post-dose (3.1 ± 0.25 mg/dL) compared to pre-dose (3.6 ± 0.22 mg/dL). In addition, urinary urate/plasm creatinine excretion ratio significantly increased ($p<0.05$) 5 hours post-dose with peak excretion at 3 hour ($p <0.01$) compared to pre-dose. These results suggest that cherries may exert an acute uricosuric effect in addition to containing high levels of anthocyanin (anti-inflammatory) and ascorbic acid or vitamin C (antioxidant). Together, this may well explain the anti-inflammatory and uric acid-lowering effects associated with cherries

consumption during gout attacks.

From an evolutionary perspective, it could be theorized that uric acid became the endogenous antioxidant as a compensatory mechanism when vertebrates lost their ascorbic acid synthesizing-gene. Moreover, the loss of uricase enzyme in humans, which converts uric acid to allantoin in other species, possibly made uric acid critical for survival. Therefore, the effect of vitamin C ingestion on uric acid metabolism has been explored in patients with hyperuricemia or gout. Indeed, it has been shown that ingestion of vitamin C especially at higher doses could enhance uric acid excretion.[38] A study used vitamin C 500mg supplement for 2 months showed a significant reduction in uric acid. Furthermore, an epidemiological study conducted in Korean Multi-Rural Communities Cohort (n=9400) identified an overall significant trend for reduced risk of hyperuricemia associated with increased dietary and total vitamin C intake. Stratified by gender, females had significant trends for reduced risk of hyperuricemia with either source of vitamin C; however, males only had a significant trend for reduced risk of hyperuricemia with dietary vitamin C.[120] Although the urate-lowering effect mechanism of vitamin C remains unclear, it is hypothesized that vitamin C competes for uric acid reabsorption and possibly inhibits xanthine oxidase.

Smoking has been an established risk factor for numerous diseases and comorbidities while being a leading cause of lung cancer. Smoking has also been shown to influence uric acid levels and modulate the risk for developing gout.[121] While the exact mechanism by which smoking can affect uric acid levels remains unknown, recent studies have shown that smoking has been associated with lower SUA levels leading to reduced

risk for developing gout.[122] Specifically, analysis of 54-year follow-up data from the Framingham Heart Study involving 2279 men and 2785 women who were gout free at their first assessment was used to assess the direction and magnitude of association of smoking and onset of gout. The study concluded that smoking was independently associated with overall reduced the risk of gout incidents of by 24% (HR 0.76, [95% CI,0.59 to 0.98]). When stratified by sex, the risk reduction of gout incidence was significant in men but not in women (HR 0.68, [95% CI, 0.59 to 0.98] vs. 0.92, [95% CI, 0.6 to 1.41]), respectively.[123] Also, the study noted that SUA was generally lower in the smokers than non-smokers; however, it did not reach statistical significance. The results of this study prompt the need for understanding the mechanism by which smoking can influence SUA or development of gout. To date, the main proposed mechanism for this observation may be the inactivation of XO by thiocyanate- the end product of detoxification of hydrogen cyanide present in cigarette smoke.[124] However, the role of nicotine and its metabolites on SUA remains a hypothesis generating question.

Hyperuricemia, Gout and Cardiovascular Diseases

The association between gout and cardiovascular diseases has been observed since the late 19th century. This association became more evident in 1950s-1960s when several epidemiological studies have shown that not only hyperuricemia but the normal high SUA was also associated with incidence of cardiovascular related comorbidities.[125] However, this association remains too controversial to mandate uric acid monitoring in order to follow an individual's risk for cardiovascular disease. While there are reports that show uric acid can be an independent risk factor for developing cardiovascular disease as well

as kidney diseases [Figures 2.6-7], uric acid may not be the direct cause of kidney disease but it may however lead to the development of hypertension which has a greater impact on the kidney than uric acid itself. Therefore, the causal relationship of uric acid and CVD requires a reappraisal and consideration of studies to show the direct effect of uric acid on CVD and kidney disease.[23]

The effect of hyperuricemia on hypertension itself, has been a consistent observation from many studies involving animals and humans.[56,126-130] In addition, the parallel of the magnitude of effect hyperuricemia has on blood pressure values has been consistent for adults with pre-hypertension and essential hypertension. In one study, an elevated SUA > 5.5 mg/dL was observed in 90% of adolescents with essential hypertension whereas SUA levels were significantly lower in controls with white-coat or secondary hypertension. This finding further suggests that the development of hyperuricemia generally precedes the development of essential hypertension [Figure 2.7-8].[131]

The mechanism by which hyperuricemia can cause hypertension may involve two primary mechanisms. Animal models of hyperuricemia suggest that high SUA can cause direct microvascular changes to the endothelial cells and vascular smooth muscles as well as systemic and glomerular hypertension. In addition, high SUA is also associated with increased renin level activity which in turns causes more vasoconstriction and decreased salt excretion (Figure 2.7-8).[22] These proposed mechanisms may suggest that reversing hyperuricemia in individuals diagnosed with pre-hypertension or early onset of hypertension could achieve normal blood pressure upon treatment using urate-lowering therapy (ULT). To test whether lowering SUA could lower blood pressure, 30 adolescents

newly diagnosed with hypertension having SUA ≥ 6mg enrolled into a randomized, double-blind, placebo-controlled crossover trial.[132] The study showed using 200mg allopurinol twice daily for 4 weeks significantly lowered the casual and 24-hour ambulatory systolic and diastolic blood pressures. Specifically, the mean change in 24-ambulaotry SBP for allopurinol was -6.3 mm Hg [95% CI, -3.8 to -8.9] vs 0.8 mm Hg [95% CI, 3.4 to -2.9] for placebo. Similarly, the mean change in the 24-ambulaotry DBP for allopurinol was -4.6 mm Hg [95% CI, -2.4 to -6.8] vs -0.3 mm Hg [95% CI, 2.3 to -2.1]. However, these findings have not been replicated in large clinical trials to qualify the use of allopurinol in the manage of hypertension or treatment of asymptomatic hyperuricemia.

Although gout as an independent risk factor for CVD could remain controversial, the question to be asked is whether the management of gout using ULT could reduce CVD associated mortality. A large prospective case-matched cohort study was conducted over 6.5 years to assess the risk of death from CVD and all-cause mortality in 40,623 Taiwanese patients with gout aged ≥17 years. In addition, the study assessed the effect of ULT on mortality risk in patients with gout. [133] The study concluded that patients with gout not treated with ULT had an increased risk as high as 143% and 45% for CVD mortality and all-cause mortality, respectively. On the other hand, patients with gout treated with ULT had 71% and 53% risk reduction for CVD mortality and all-cause mortality, respectively. Furthermore, the duration of ULT was also important in predicting CVD mortality risk reduction, especially in patients using ULT >2 years. While the effect of ULT on CVD mortality did not differ across drug classes, most participants in the study

were taking benzbromarone (73%) followed by allopurinol (53%).[133]

This study highlights two critical aspects for managing patients with gout. First, the benefits of managing gout extend beyond the immediate gout therapy outcomes of reducing flares or preventing the formation of urate crystals. Second, the treatment modality for managing gout may have a little impact on CVD outcomes rather than the patients is being treated and controlled

Uric acid and Neurodegenerative Diseases

While high uric acid levels and gout are strongly associated with increased cardiovascular and endocrine comorbidities, the antioxidant effect of uric acid may have a neuroprotective effect which could explain the mutual exclusivity of gout and multiple sclerosis.[134] From an evolutionary perspective, the loss of uricase activity led to higher SUA levels, which may have had survival benefit as an antioxidant in humans and higher primates and sustaining blood pressure during evolutionary bottlenecks. This theory could provide a rationale for mammals having a very low SUA levels relative to humans. Furthermore, epidemiological studies have shown that individuals with that low SUA levels have a higher risk and faster progression of Parkinson's disease (PD).[135]

Levels of SUA have been shown to be influenced by genetic variabilities, primarily SNPs, within key uric acid transporters.[80,81 83]Given the normal physiological role of uric acid as a natural antioxidant, the Mendelian Randomization approach would suggest that more patients with neurodegenerative diseases would be carriers of risk alleles associated with lower SUA than controls. To address and test this hypothesis, a case-control study design was used to estimate the genetic risk score of previously reported eight loci in

patients with PD (n= 1061) and without PD (n=754). The study concluded that male patients with PD had a lower SUA than controls (5.61 vs. 6.27, p=0.04). In addition, the study identified that patients with > 9 genetic risk score (range 0-16) associated with low SUA, had OR of 1.55 (95% CI, 1.10-2.18) compared to controls with 2-7 risk alleles to have PD.[136] In contrast, SUA levels did not show a significant effect on the development of dementia in patients with PD.[137]

Additionally, a study by Lu et *al* [138] evaluated the potential role of gout and the risk of developing Alzheimer Disease (AD) in the general population using matched cohort design for age, sex, entry-time, and body mass index from an electronic database representative of the UK general population. The study identified that gout was significantly and inversely associated with new cases of AD. After a median follow-up of 5 years, HRs for developing AD among patients with gout were 0.71(95% CI, 0.62- 0.80), and 0.76 (95% CI, 0.66- 0.87) in both univariate and multivariate analyses, respectively. With evidence suggesting that uric acid is a neuroprotective, one may postulate that using uric acid therapeutically in neurological disease or cerebral injuries may contribute positively to the overall therapeutic outcomes. Although some research is ongoing to investigate the use of uric acid in established neurodegenerative diseases, the combined use of uric acid with the thrombolytic agent- alteplase in acute ischemic stroke has been conducted. Specifically, a study in patients with ischemic stroke assessed the proportions of patients with excellent outcomes at 90 days after receiving uric acid versus placebo in addition to alteplase. Although the primary outcomes occurred more in the uric acid group versus placebo (39% vs. 33%, p=0.099), other subgroup analyses showed marginal

significant effect favoring the co-administration of uric acid with alteplase therapy.[139]

Pathophysiology of Gout

Gout is not a stagnant disease but rather progressive and, if not controlled, can result in greater damage to the tissues and joints. However, gout is divided into multiple stages depending on the disease presentation. Generally, gout is preceded by asymptomatic hyperuricemia, which is followed by the first onset of acute gout attack. Further, the acute gout attack can be recurrent attacks interspersed by intercritical asymptomatic hyperuricemia. Ultimately, if not controlled, gout can progress to a symptomatic tophaceous gouty arthritis and enhanced risk of forming uric acid kidney stones.

Gout is the most common arthritic inflammatory disease caused by the deposition of Monosodium Urate (MSU) crystals in the joints as well as soft tissues. While the concentration of SUA is a critical determinant of MSU crystals formation, the tissue environment also plays a key role in their precipitation. For instance, tissues and joints that are not highly perfused may result in a local environment whereby a reduction in temperature at that joint may enhance MSU formation.[140] Moreover, the existence of an ongoing trauma or injury at those joints or tissues around them can augment the risk of crystal formation. These observations are supported by the observed association between osteoarthritis and gout.[141] Therefore, the preemptive management of gout flares in patients with a history of joint disease can incur clinical benefit by deterring new onset of joint-related or musculoskeletal diseases. Furthermore, the optimal management of uric acid levels of patients with existing joint disease can further reduce and prevent tissue inflammation and ward off further joint damage or the development of gout later in life

[Figure 2.3].

The deposition of the MSU is dependent on the solubility and saturation levels of the ionic product of sodium and SUA. Generally, SUA concentrations that exceed 6.8mg/dL are considered critical for MSU crystals formation and deposition within and between joints.[140] Monosodium urate crystals tend to primarily affect the distal joints. For example, the metatarsal phalanges tend to be the most targeted joints for crystals deposition causing tophi.[140] While the distal joints are the primary sites for MSU deposition, the extra-articular manifestations and the soft tissues including kidney, synovial membrane and the vasculature system share the burden of elevated levels of SUA causing interstitial nephritis and synovitis, respectively. MSU crystals tend to reside in the site of origin and within the synovial fluids. However, the dissolution or shedding of the MSU crystals is a major trigger for the immunological response characterizing the swelling, erythema, and pain associated with acute gout attacks [Figure 2.3]. This response is mediated by the monocytes' phagocytic activity towards the MSU crystals consequently activating NALP3 also called cryopyrin. The release of NALP3 inflammasome complex triggers the release of IL-1 and other cytokines subsequently the infiltration of neutrophils. [142]

Pharmacotherapy of Gout and Hyperuricemia

In clinical practice, asymptomatic hyperuricemia is not usually treated and no FDA approved drugs have been indicated for its management. Nonetheless, since elevated SUA is a precursor to gout, the pharmacotherapy associated with managing patients with gout naturally targets SUA levels as the key therapeutic outcome. The treatment goals for managing a patient with gout include achieving SUA <6mg/dL, reduce the risk for new

onset or recurrence of acute gout attacks, and optimal management of other diseases or comorbidities. Additional goals include ensuing dietary and lifestyle changes, improve quality of life, and ultimately prevent further joint damage due to chronic gouty arthritis.[143] Acute gout attacks are extremely painful and often debilitating for the patient and associated with a high indirect societal cost (lost days of work, physical immobility, reduced quality of life). While the frequency of gout attacks is a marker for how gout is controlled, the immediate resolution of acute gout symptoms is of a paramount goal to patients with gout and the society at large.[144] A pictorial summary of the drugs used to manage acute gout attacks and longer-term management of gout is shown in Figure 2.9.

Management of Acute Gout Attacks

In general, there are several approaches to managing acute gout attacks. Preference is given to those therapies thought to be the most effective, tolerated and with the least side effect profile. Therefore, the general approach involves an individualized assessment of the benefits and risks of each of the following drug therapies.

The first line of drug pharmacotherapies used to manage acute gout attacks are the non-steroidal anti-inflammatory drugs (NSAIDs), followed by colchicine followed or corticosteroids. Although these drugs are equally efficacious, the selection amongst drug classes is patient-specific and possibly cost driven. For instance, consideration of the patient's current medication list and existing comorbidities as well as drug cost are critical determinants for choosing the right drug for the right patient to optimize response and resolve existing symptoms.[145,146] Further discussion on the therapeutic options within each class and some key considerations while using these drugs for managing patients with acute gout attacks is summarized in the following sections.

NSAIDs have been used extensively in managing a wide range of symptoms of pain and inflammatory disease states; acute gout attack is a classic indication for the use of NSAIDs to control pain and reduce inflammation. The mechanism of action of NSAIDs is to inhibit the Cyclooxygenase (COX) enzyme, which is responsible for the conversion of arachidonic acid to prostaglandins, which play major role in the pain signaling cascade during acute gout attack. Consequently, targeting the prostaglandins formation cascade presents as a reasonable mechanism to reduce the pain associated with gout attack. Although NSAIDs are effective and less costly drugs to control pain, they are associated with some adverse events in a select patient population. Therefore, assessment of the patient's risk for CVD, gastrointestinal (GI) bleeding, chronic kidney disease, existing peptic ulcer, or the use of concomitant drugs that would potentiate the risk for bleeding are important to consider when selecting an NSAID. Furthermore, though most NSAIDs are equally effective, some NSAIDs tend to have an additive benefit to use amongst patients with gout. For instance, indomethacin has been shown to possess some uricosuric effects relative to piroxicam.[147]

Moreover, most the NSAIDs (ibuprofen, naproxen, diclofenac, celecoxib) are substrates for hepatic metabolism via the CYP2C9. Therefore, knowledge of CYP2C9 genetic status of reduced function *may* further guide the selection between different NSAIDs while minimizing the risk for GI bleeding.[148] However, it must be borne in mind that the prescribing habits of clinicians change over the course of time and by region, which could explain the declining trend of NSAID prescriptions whereas colchicine is trending up, in part due to the cardiovascular risks associated with NSAID use.

Furthermore, celecoxib is the only NSAID on the market that selectively inhibits COX-2 enzyme, which may be associated with decreased risk of GI bleeding for patients who are not candidates for colchicine or long-term use of corticosteroids. Additionally, the co-prescribing of proton pump inhibitors with long-term use of NSAID can further reduce the risk of GI bleeding.

Colchicine is an alkaloid derived from extracts of meadow saffron, which was discovered in the early 1700 AD to provide some relief during gout attacks. The exact mechanism of colchicine to alleviate pain is by disrupting the polymerization and mobilization of the microtubules subsequently interfering with the neutrophil functions, lysosomal degranulation, and leukocyte chemotaxis. Furthermore, colchicine suppresses the MSU crystals-induced NALP3 inflammasome driven capsase-1 activation and interleukin (IL)-1 β processing. These effects collectively suppress the inflammation response and the infiltration of the immunological cells at the site where MSU crystals triggering attacks. [149]

The dosing regimen of colchicine has undergone major changes as a result of the findings from the randomized, double-blind, placebo-controlled, parallel-group, dose-comparison colchicine study, which assessed the safety and efficacy of a low dose colchicine versus the high dose colchicine.[150] The study identified that the low dose of colchicine defined as 1.2mg at the onset of the gout attack followed by 0.6mg an hour later was as equally effective as the high dose defined as 1.2mg at onset of gout attack followed by 0.6mg hourly for a total of 6 hours. Furthermore, the lower dose of colchicine had lower incidences of adverse events than those associated with the high dose.

Colchicine is a p-glycoprotein substrate, metabolized in the liver by CYP3A4, and cleared by the kidney; therefore, assessment of liver function and kidney function as well as the presence of strong CYP3A4 inhibitors (clarithromycin, cyclosporine, verapamil, ketoconazole) are important for dosing adjustment. On one hand, colchicine is known to have a narrow therapeutic index, significantly associated with gastrointestinal toxicities (nausea, vomiting, diarrhea) and potentially myotoxic (muscle weakness, myalgia, rhabdomyolysis). These unique characteristics of colchicine warrant careful assessment of potential adverse events to minimize the risk of colchicine toxicity when co-administered with drugs that are known to be CYP3A4 inhibitors and/or p-glycoprotein substrates.[146,149,151] On the other hand, the long-term use of colchicine has been significantly associated with reduced risk of myocardial infarction in patients treated for the duration of 3 years with relative risk of 0.20 [95% CI, 0.07 to 0.57] though it was not significantly associated with reduced all-cause mortality.[152]

Corticosteroids (CS) are effective option in the management of acute gout attacks, especially if the patient cannot tolerate oral NSAIDs or colchicine. The routes of administration of CS (oral, parenteral, intra-articular) further allow their use in various clinical settings as well as disease states. The main mechanism of action of CS is the inhibition of the phospholipase A2, thereby blocking the eicosanoids production, leukotriene synthesis, and various leukocyte inflammatory functions. While large randomized control trials are lacking, small studies have consistently shown comparable efficacy of using either oral NSAIDs or corticosteroids in resolving acute gout attacks. Intra-articular triamcinolone and methylprednisolone, however, represent unique options

for patients with up to 2 joints experiencing inflammation. Obviously, intra-articular administration carries a higher risk of unmasking latent infection and one must rule out septic arthritis is needed before injection. [149]

Canakinumab is an approved Interleukin-I (IL-1) inhibitor representing a unique option for patients with Systemic Juvenile Idiopathic Arthritis (SJIA). Although it is not FDA approved for use in gout attacks, it presents a possible alternative for patients experiencing frequent gout attacks and intolerant or refractory to other therapeutic options, or not candidates for colchicine, CS, or NSAIDs.[153] Given the shared inflammation pathway associated with both SJIA and MSU-induced inflammation, it is believed that canakinumab could be as effective if not superior to other agents used to manage acute gout attack.[149] In fact, two published phase III studies[154] compared canakinumab (n=230) and triamcinolone (TA)(n=236) in patients with acute Gout flare. Compared to TA 40mg intramuscularly (IM), canakinumab 150mg subcutaneously was found not to be only significantly associated with reduced pain score, but also delayed the time to first new flare and reduced the risk of new flares by 62% versus TA 40mg IM (HR: 0.38; 95% CI 0.26 to 0.57). However, adverse events reported more frequently with canakinumab included infections, low neutrophil count and low platelet count. Summary of pharmacological options for the management of acute gout attack are listed in Tables 2.8-9.

Long-term Management of Gout

Lowering serum urate could be achieved via two different mechanisms. The first mechanism is to decrease SUA production and/ or enhance UA excretion. The second mechanism is to prevent UA reabsorption. In either case, a goal of SUA < 5-6mg/dL ,

depending the severity of gout, may not be achieved using only one approach which prompts the need for a combined approach. Therefore, understanding the mechanism of actions of these therapeutic modalities can enhance the selection of effective drug regimens to reach SUA goal.[143] Dosing and considerations of selecting ULT are summarized in Table 2.10.

Allopurinol is a purine analog xanthine oxidase (XO) inhibitor and has been extensively used since 1950s to manage patients with gout and remains the most prescribed drug amongst 96% of patients with gout. [155] Allopurinol is rapidly absorbed from the GI track resulting in a bioavailability of 80% with a range of 67-90%. Allopurinol has a short half-life (1-2 hours), however, the main urate-lowering activity is attributed to the active metabolite oxipurinol, which has a much longer half-life (12-23 hours) than allopurinol.[156] Oxipurinol is mainly eliminated by the kidney and therefore changes in kidney function or polymorphisms in the transporters responsible for oxipurinol disposition may greatly explain the wide range seen in the oxipurinol half-life. Based on the oral bioavailability and the molecular weights of allopurinol and oxipurinol, it is estimated that a 100mg of allopurinol results in 90mg of oxipurinol.[156] The maximal decrease of urate following a single dose of allopurinol is reached between 6-24 hrs post-allopurinol dose. With XO being the rate limiting step in forming uric acid, it is the major metabolizing enzyme for forming oxipurinol from allopurinol. Furthermore, both allopurinol and oxipurinol are inhibitors of XO. On the other hand, a growing evidence supports that allopurinol is also a substrate for the aldehyde dehydrogenase enzyme while being also a substrate for the XO enzyme. This may be explained by the lack of XO

enzyme saturation with chronic dosing of allopurinol and the high variability of the urate-lowering response to allopurinol.[156]

Although it is the most commonly prescribed urate-lowering therapy drug with clinically established efficacy, allopurinol is significantly associated with a Severe Cutaneous Adverse Reactions (SCARs), which include hypersensitivity syndrome, Stevens-Johnson Syndrome, and toxic epidermal necrolysis. These serious and sometimes fatal adverse events have been linked to a genetic variant within the Human Leukocyte Antigen (HLA) B noted as HLA-B*58:01.[157,158]

The HLA-B is considered part of the Major Histocompatibility Complex-I (MHC-I), which plays a key role in the immune system response to present endogenous and exogenous bound peptide antigens to T cells. HLA-B is considered the most polymorphic gene in the human genome with more than 1500 alleles to date to present wide variety of "self" and "non-self" peptides. Carriers of this genetic variant, HLA-B*58:01, are at a much higher risk for allopurinol induced SCARs compared to non-carrier. Therefore, the use of allopurinol in these patients is contraindicated. The prevalence of this genetic variant is markedly higher in Asian subpopulations mainly the Korean, Han Chines, and Thai descents. Although allopurinol induced-SCARs are rare with estimated risk of 0.1 to 0.4%, it is one of the most serious adverse events with mortality rate of up to 25%. While there are therapeutic alternatives for allopurinol, the use of a prospective screening for HLA-B*58:01 carrier status can efficiently strategize the utilization of available urate lowering therapy while preventing major adverse effects that can be fatal in some patients.[159]

Moreover, the risk for these serious cutaneous adverse events has also been associated with non-genetic factors. These factors include the dose initiated, titration period, and kidney function. Though the presence of the risk allele should be sufficient for the decision of not prescribing allopurinol, the knowledge of the patient's genotype is not commonly available at initiation of allopurinol therapy. Alternatively, it was suggested that starting the patient on the lowest dose possible of allopurinol (100mg) daily while monitoring for adverse events and titrating slowly (every 2-4 weeks) to SUA goal can dramatically reduce the risk of developing SCAR.[153] Fortunately, patients who are carrier of the risk allele of HLA-B*5801 can be alternatively treated with febuxostat.

Febuxostat is a non-purine analog XO inhibitor and effectively lowers SUA production. Unlike allopurinol, febuxostat is not associated with the risk for developing SCAR and non-selectively inhibits both isoforms of the xanthine oxidoreductase. In fact, in a study of 756 patients with gout and SUA> 8mg/dL, 53% of patients(n=255) treated with 80mg febuxostat and 62% of patients (n=250) treated with 120mg febuxostat have achieved SUA<6 mg/dL compared to 21% of patients (n=251) treated with 300mg allopurinol ($p<0.001$).[160] However, the febuxostat arm had a higher incidence of cardiovascular related mortality. Another key difference between febuxostat relative to allopurinol is the route of elimination. Febuxostat is mainly metabolized in the liver via UGT1A2, whereas oxipurinol is mainly cleared through the kidney unchanged. This key difference in elimination presents as a reasonable therapeutic alternative in patients with impaired or declining kidney functions.[161] On the other hand, febuxostat has been also associated with elevated liver enzymes; therefore, regular monitoring of liver function is

recommended. Also, it must be born in mind that febuxostat is far more expensive than allopurinol which is a limiting factor for its clinical use.[161]

Although the available XO inhibitors can effectively reduce SUA, many patients receiving these drugs fail to achieve their SUA target goal. This phenomenon could be explained by three possible mechanisms. One of the major reasons is compliance to urate-lowering therapy, which is a major area for improvement.[162,163] The second reason could be attributed to the fact that most patients with hyperuricemia are UA underexcreters rather than overproducers, which is not accounted for in the diagnosis of gout or treatment guidelines.[164] The third reason could be due to the genetic variabilities within the transporters or metabolizing enzymes influencing the disposition of urate-lowering drugs.[86,165,166] Therefore, assessment of the etiology and classification of hyperuricemia could further enhance the diagnosis and selection of the appropriate therapeutic option.

Evidently, the use of XO inhibitors is extremely effective in managing patients with UA overproducer status. However, allopurinol is a unique ULT option due to oxipurinol, which can work by inhibiting XO as well as competing with UA reabsorption through the same transporter making allopurinol the first line drug of choice due to its established efficacy, low cost, and patient safety.[153]

Probenecid was the first prototype uricosuric drug to come to market over 60 years ago, to manage patients with chronic gouty arthritis. The exact mechanism of probenecid is mediated by blocking uric acid reabsorption via the OATs and URAT1 transporters on the apical side of the proximal convoluted tubule. Unlike other uricosuric agents, probenecid could increase uric acid clearance by 4-6-fold at 1-2gm per day. This dramatic

increase in uric acid clearance can enhance the risk of uric acid nephropathy, uric acid nephrolithiasis and uric acid urolithiasis.[167] Consequently, probenecid is not recommended in patients with CrCl < 30mL/min or have a history of kidney stones. However, using daily divided probenecid doses, increase liquid consumption and adequate urine flow while alkalinizing the urine can greatly decrease the risk of uricosuria associated complications.

The efficacy of probenecid as a monotherapy in patients with primary gout has been clinically debated. Specifically, patients with reduced kidney function or chronic kidney disease are at a higher risk for developing more probenecid-related side effects compared to those with normal kidney function treated with probenecid. To investigate this debate further, a retrospective observational study assessed the efficacy and tolerability of probenecid as monotherapy (n=30) or in combination with allopurinol (n=27) for the treatment of gout. The study found that the proportion of patients achieving target SUA (< 6mg/dL) in the monotherapy group (33%) and in the combination therapy (37%) were not different (p=0.75). Further, the adverse events attributed to probenecid did not significantly differ between the patients with eGFR $\geq$ 50 ml/min/1.73 m^2 19% (8/42) versus eGFR < 50 ml/min/1.73 m^2 13% (2/15).[168]

The interaction of co-administering probenecid with allopurinol and the overall impact on SUA reduction in healthy volunteers and patients with gout was also evaluated. Consistently, those studies concluded that although probenecid increased the renal clearance of oxipurinol, the net effect of SUA reduction in the combination therapy was significantly higher than probenecid alone.[169,170] In summary, probenecid is moderately

effective in lowering SUA in patients with declining kidney function and may be used as monotherapy in patients who are intolerant to allopurinol. Furthermore, probenecid is an effective add-on treatment for patients with gout not achieving their SUA goal by using allopurinol alone.

Lesinurad is a uricosuric drug that was approved by the FDA in 2015. It inhibits uric acid reabsorption by blocking the URAT1 and OAT4 transporters thus enhancing uric acid renal clearance. Lesinurad is indicated in combination with XO inhibitor for the treatment of hyperuricemia associated with gout in patients who have not achieved target SUA levels with XO inhibitor.[171] At 200 and 400 mg doses added to allopurinol, lesinurad significantly increased proportions of patients achieving SUA target versus allopurinol-alone therapy by month 6 (55.4%, 66.5% and 23.3%, respectively, $p<0.0001$).[172] On the other hand, renal-related adverse events occurred in 5.9% of lesinurad 200 mg + allopurinol group, 15.0% of lesinurad 400 mg + allopurinol group and 4.9% of allopurinol-alone group, with serum creatinine elevation of >1.5x baseline in 5.9%, 15.0% and 3.4%, respectively.[172]

Consequently, the maximum FDA approved dosage is 200mg daily and to be given with allopurinol or febuxostat. The dose should be taken in the morning with food and water. Also, patient should be instructed to remain hydrated to avoid risks of acute renal failure or nephrolithiasis. Lesinurad can be cautiously used in patient with eCrCL < 60mL/min but not to be used in patients with eCrCl <45mL/min. Lesinurad is a CYP2C9 substrate therefore genetic polymorphisms in CYP2C9 or co-administration with CYP2C9 substrate drugs can result in drug-drug interactions. Specifically, one might expect elevated levels

of lesinurad in individual with genotype of CYP2C9*2 and *3. Furthermore, lesinurad should not be used with allopurinol at doses below 300mg/day or 200mg/day if eCrCl is < 60mL/min. Moreover, if the xanthine oxidase inhibitor therapy was interrupted, lesinurad should be also interrupted.

Fenofibrate is an approved lipid lowering drug and has been associated with SUA reduction of 15% and up to 46% in patients with gout with hyperlipidemia.[173] The presence of hyperlipidemia in patients with gout is highly prevalent which makes fenofibrate an optimal choice for patients with hyperlipidemia suffering from gout. [174] Although the use of fenofibrate as uricosuric agent is not FDA approved, current guidelines for the management of gout recommend its use in patients who may benefit from further reduction of SUA, especially with dyslipidemia.[143,153] The urate-lowering effect of fenofibrate is believed to be mediated through URAT1 transporter inhibition by fenofibric acid.[175] Moreover, some statins such as pravastatin and atorvastatin have shown some additive benefits of lowering uric acid, improving renal function while being used to lower cholesterol.[176] These urate-lowering benefits associated with these drugs can enhance the co-prescribing habits when patients need a further urate-lowering reduction.

Losartan is an approved antihypertensive agent and has shown to reduce SUA by 6% and up to 23% in patients with a select URAT1 genotype through enhancing UA excretion.[173,177,178] Thus, the use of losartan can complement the effect of other ULT while controlling for high blood pressure which is a common comorbidity amongst patients with gout.[174] The exact mechanism by which losartan enhances UA excretion is not clearly understood; however, it is presumed that losartan blocks the URAT1 transporter while

alkalinizing the urine which further enhances UA excretion. It is worth noting that the specific selection of an antihypertensive to manage a gouty person with hypertension can both be complementary to treating both conditions or counterproductive, at least to the management of hyperuricemia or gout. This represents an opportunity to clinicians for comprehensive reviews of their patients being managed for these two conditions. Other antihypertensive class such as the calcium channel blocker, amlodipine has shown an additive benefits by lowering uric acid while being used to lower high blood pressure in a select patient population.[108] Study synopses that investigated the effects of losartan and fenofibrate on uric acid levels are summarized in Table 2.10.

Pegloticase is the human recombinant form of the uricase enzyme, which has been developed and approved in the management of advanced cases of tophaceous gouty arthritis. Pegloticase is an intravenous uricolytic enzyme that converts UA to the water-soluble form, allantoin. Although it rapidly and effectively reduces SUA, pegloticase has been associated with some serious adverse events. For instance, pegloticase antibodies have been developed, infusion reaction has occurred, and anaphylaxis events have been reported. Moreover, the administration of pegloticase in patients with the glucose-6-phosphate dehydrogenase (G6PD) deficiency is contraindicated due to the increased risk of hemolysis and methemoglobinemia. Therefore, knowledge of G6PD status is warranted especially in patients with African and Mediterranean ancestry.

Canagliflozin is a sodium glucose cotransporter 2 (SLGT2) inhibitor and an approved antidiabetic drug, which has been associated with SUA reduction with up to 13%.[179] Furthermore, a study has shown the SLGT2 inhibitor, luseogliflozin, to be also

associated with SUA reduction by up to 25%. Although the mechanism by which canagliflozin reduces SUA is unknown, luseogliflozin has been shown to significantly increase urinary excretion of uric acid in healthy subjects and by modulating *Xenopus* oocytes expressing GLUT9-2 transporter.[180] Summary of all pharmacologic treatments used for the long-term treatment of gout attack is listed in Table 2.11. Additionally, dietary recommendations for long-term management of patients with gout are summarized in Table 2.12.

Hyperuricemia and Gout Perspectives

Relative to non-primates, humans have much higher levels of uric acid due to the lack of the enzyme uricase which is responsible for converting uric acid to allantoin. This physiological increase in concentration of uric acid has led to the belief that uric acid played major physiological and protective roles in humans to enhance survival and protect against diseases that could be human specific. One of the key roles of uric acid is its antioxidant effect which helps the body to detoxify from oxygen free radicals and peroxynitires that are formed because of the XO enzyme activity. These radicals if not intercepted, they could cause major damage to tissues and trigger major inflammatory response. Now that uric acid is an antioxidant which allows us to propose the question of why and how high uric acid levels would be strongly associated with a more of a pro-inflammatory effect by causing joint damage, endothelial dysfunction, and kidney damage? One of the proposed theories is that uric acid remains an important extra-cellular antioxidant, but high concentration of uric acid above solubility levels results in the two major events. First, the formation of MSU which is recognized by the body as a foreign

object triggering a strong inflammatory response known as acute gout attack. Second, the infiltration of uric into soft tissues because of exceeding the saturation levels would result in a direct damage to those tissues while presenting itself as an invader to the intracellular defense mechanism causing major damage to both tissues and organs.

The optimal management of gout although attainable in a primary care setting, many patients with gout go untreated or fail to reach their goal of SUA < 6mg/dL.[76,181] Furthermore, patients with gout experiencing recurrent acute attacks may require a more aggressive goal of SUA < 5mg/dL. Moreover, patients with gout although maybe prescribed ULT, adherence rate to gout medications is known to be lower compared to other medications. This is partly caused by the inter-critical periods of asymptomatic hyperuricemia, which in turns do not result in gout attacks. Therefore, raising awareness about gout and an extensive as well as a thorough patient education are key steps to optimize patient's adherence. On the other hand, educating physicians and clinicians on the optimal dosing of ULT can help patients reaching their target goal of SUA as well as prevent or minimize the risk of acute gout attacks. Other strategies have been proposed to monitor adherence to ULT to rule out patients who are refractory to one therapy or the other; however, these strategies heavily rely on measuring drug levels as an assessment of adherence and mainly allopurinol. The response to allopurinol is known to possess a markedly high intersubjective variability with little research being done on understanding the root cause of this variability. Further research is needed to prospectively determine the different covariates associated with allopurinol response while better our understanding of the differential prevalence of risks factors associated with hyperuricemia across different

racial groups.

Figure 2:1 The Prevalence of gout across different age groups and gender between 1990-1999 using managed care data[62]

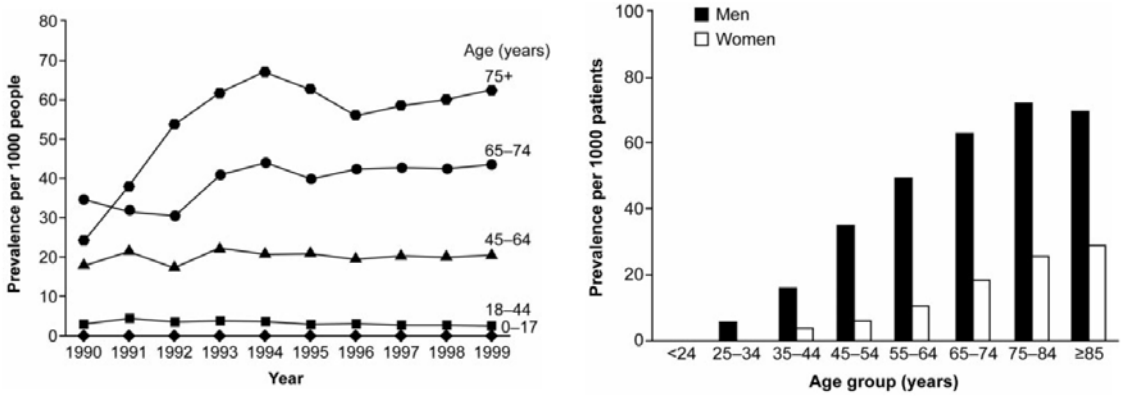


Figure 2:2 Schematic diagram of the physiological formation and elimination of uric acid in human and the sites of action for urate lowering therapies

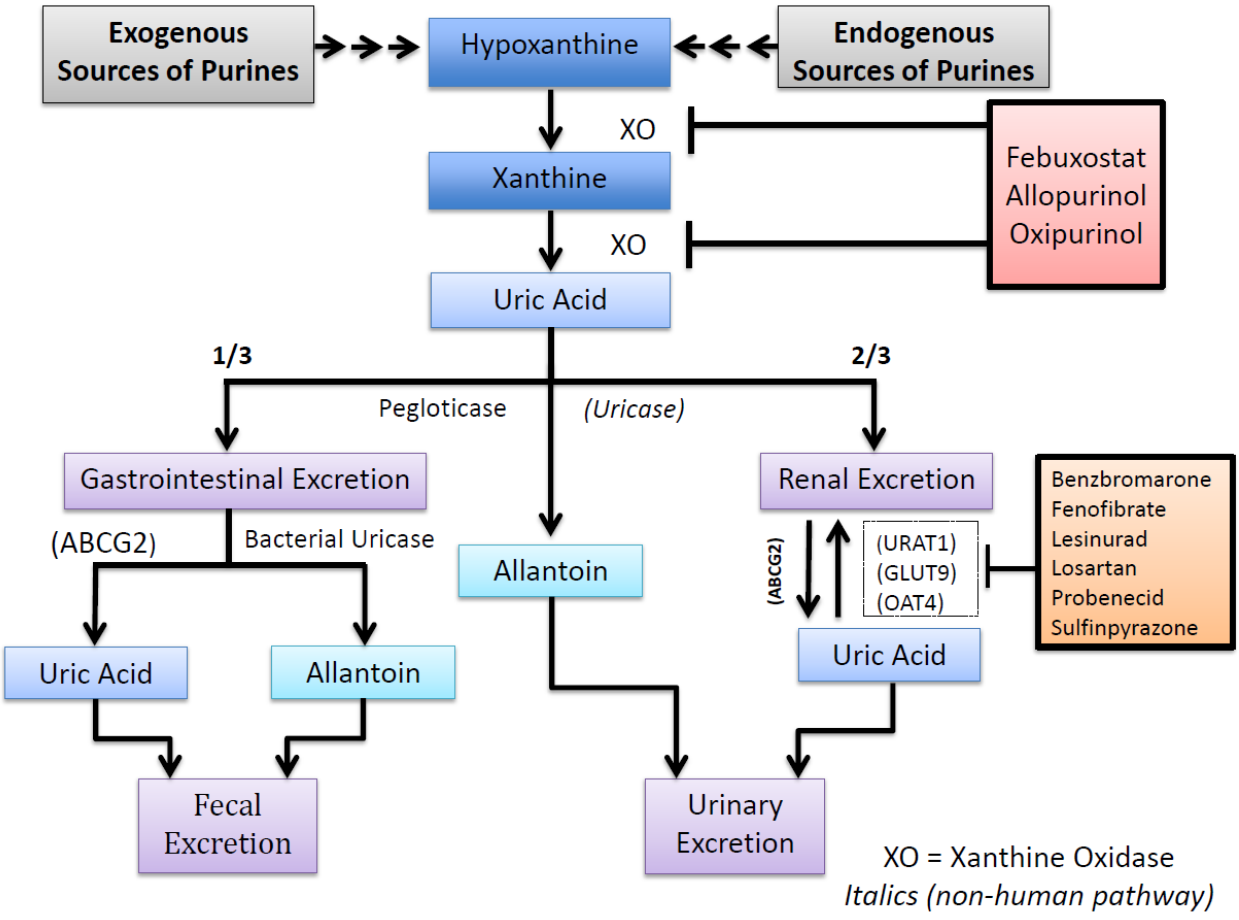


Figure 2:3 Schematic diagram of the processes involved in Monosodium Urate Crystal formation and consequent events associated with both management approaches

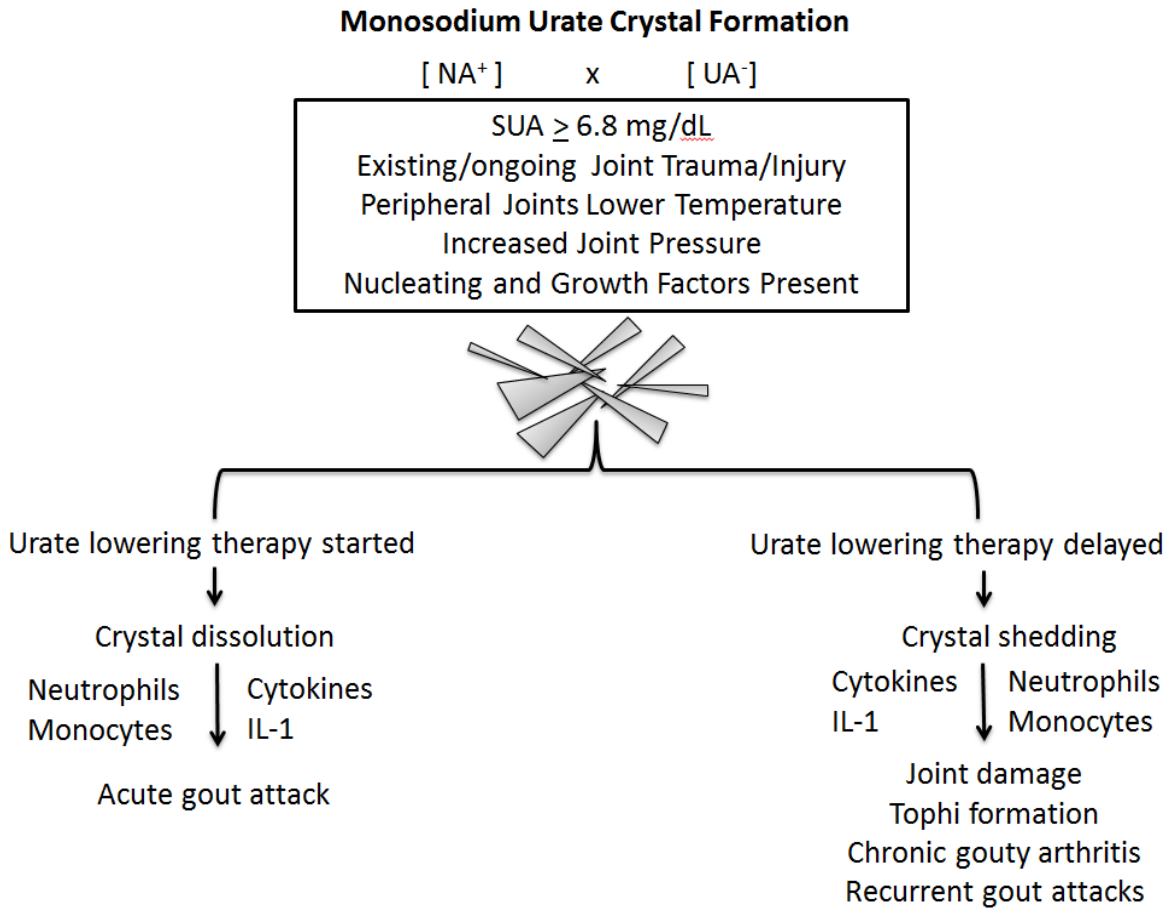


Figure 2:4 Schematic diagram the renal handing of uric acid and urate transportome[182]

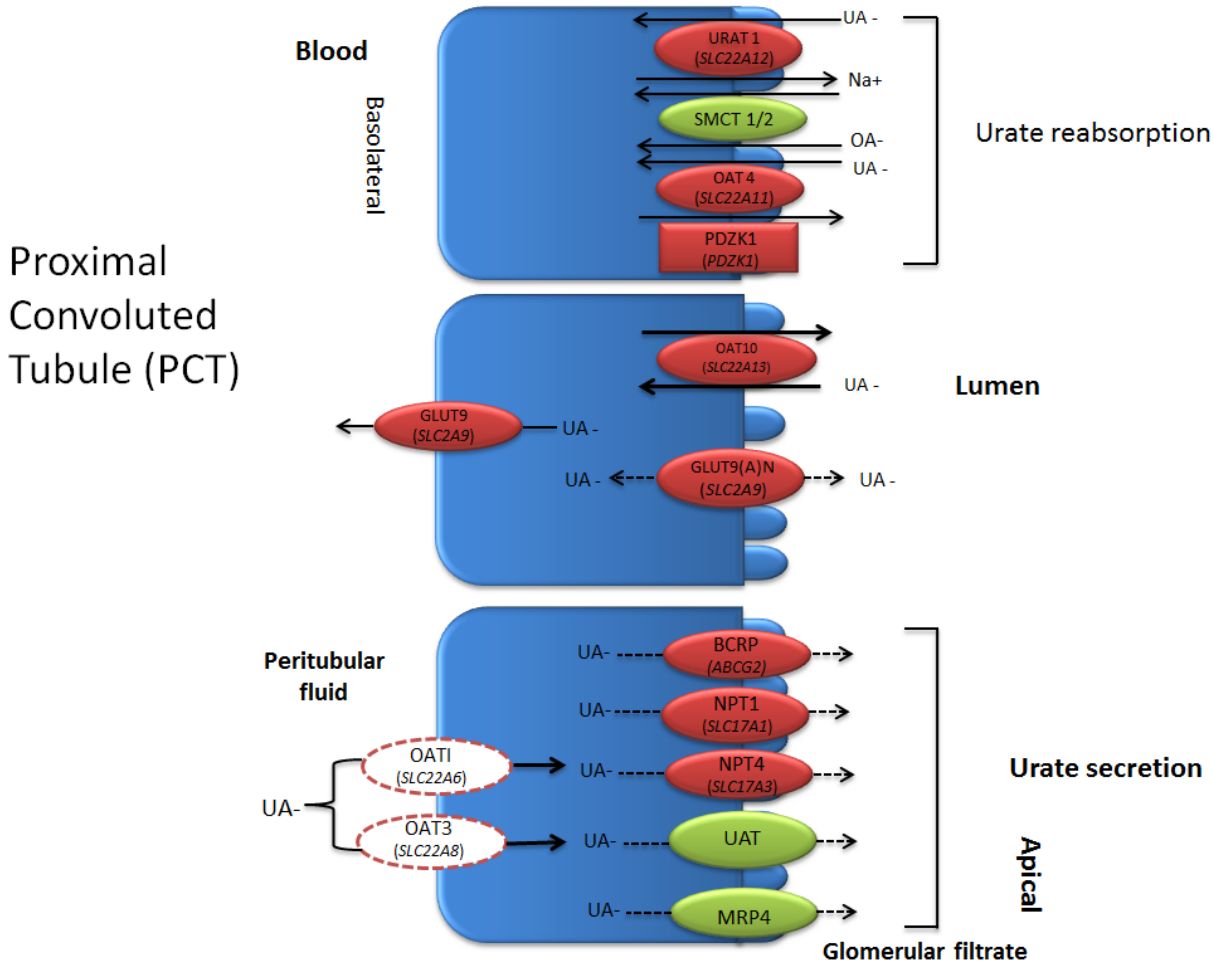


Figure 2:5 Schematic pathway for purines metabolism.
This figure also highlighted mechanism by which high consumption fructose can lead to high production of uric acid which leads to a higher depletion of the ATP leading to more IMP and inosine to form uric acid. The depletion of the ATP also stimulates the de-novo synthesis of ATP which is also a major source for purine metabolism.

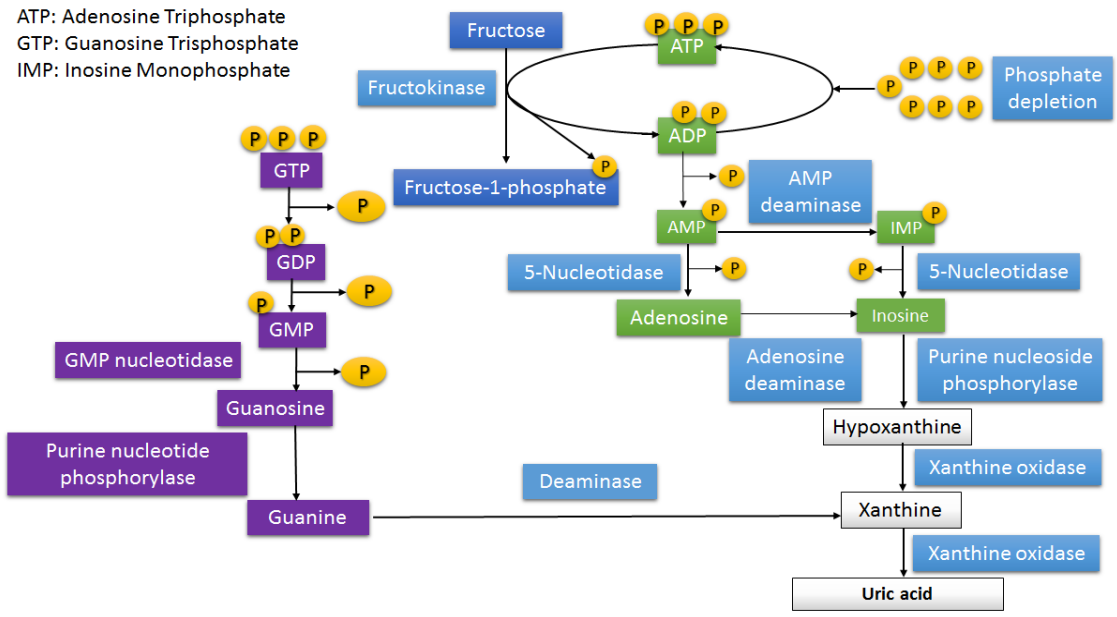


Figure 2:6 Schematic diagram of the uric acid 4-Components Theory.
This theory explains the high efficacy of the kidney in reabsorbing uric acid via multiple processes which explains the small fractional urinary excretion of uric acid.[74]

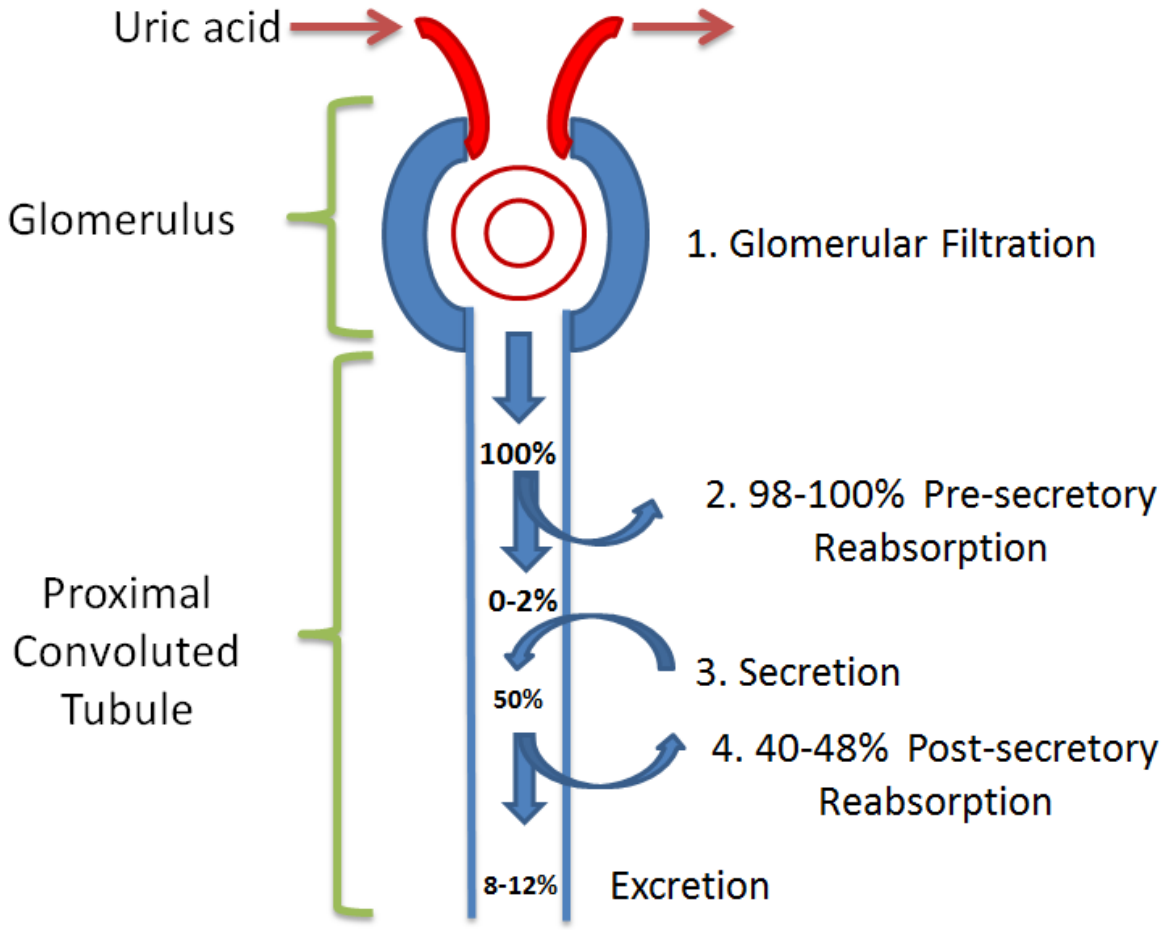


Figure 2:7 Proposed model for cardiovascular risks associated with hyperuricemia.
The figure depicts the three possible mechanisms by which hyperuricemia could be contributing to the development of cardiovascular diseases as well as the role of cardiovascular disease or the management of these condition could be also contributing to the development of hyperuricemia.

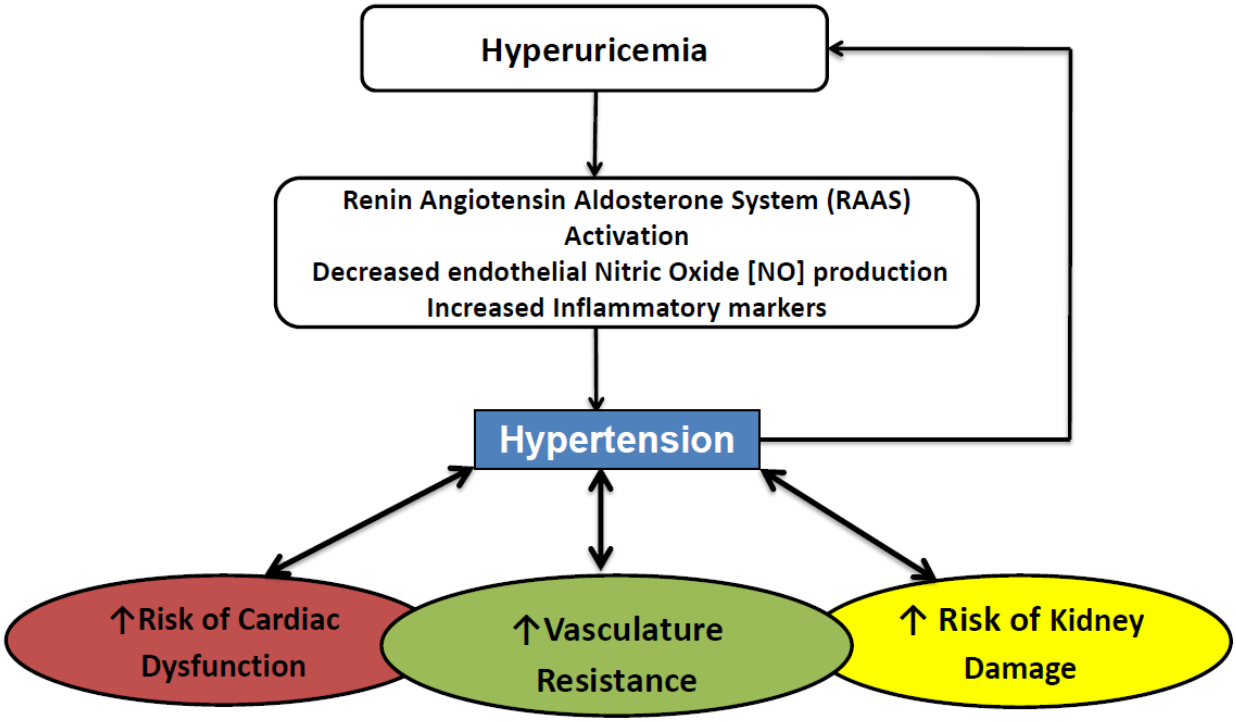
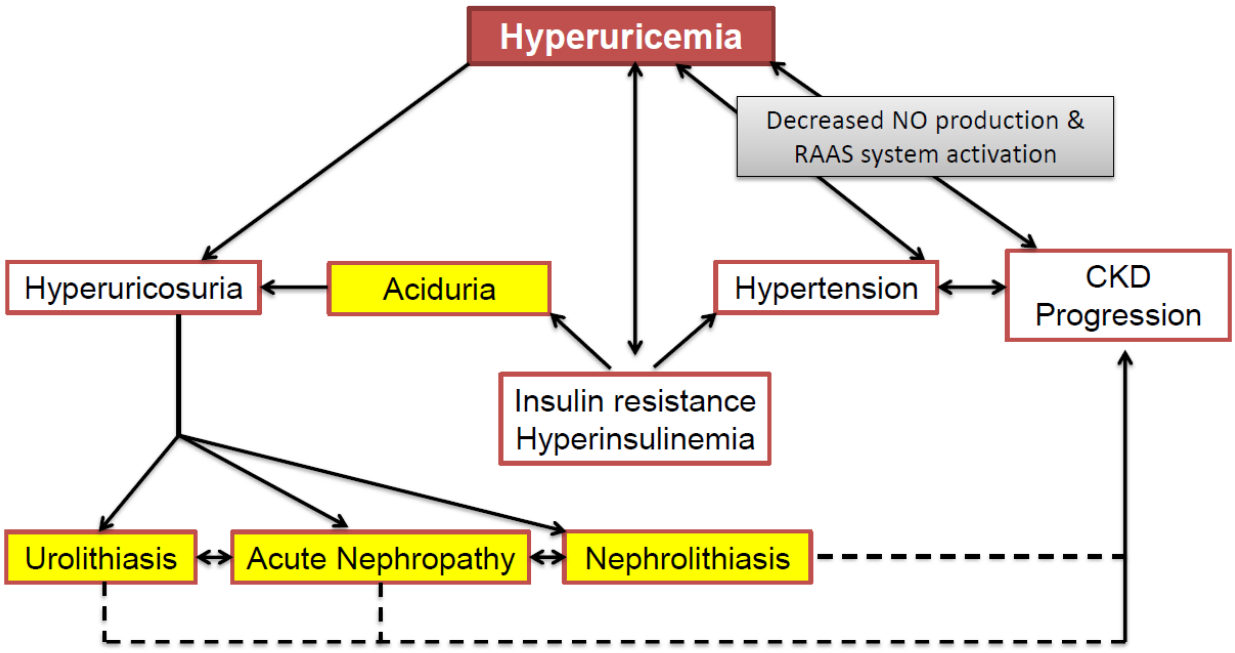
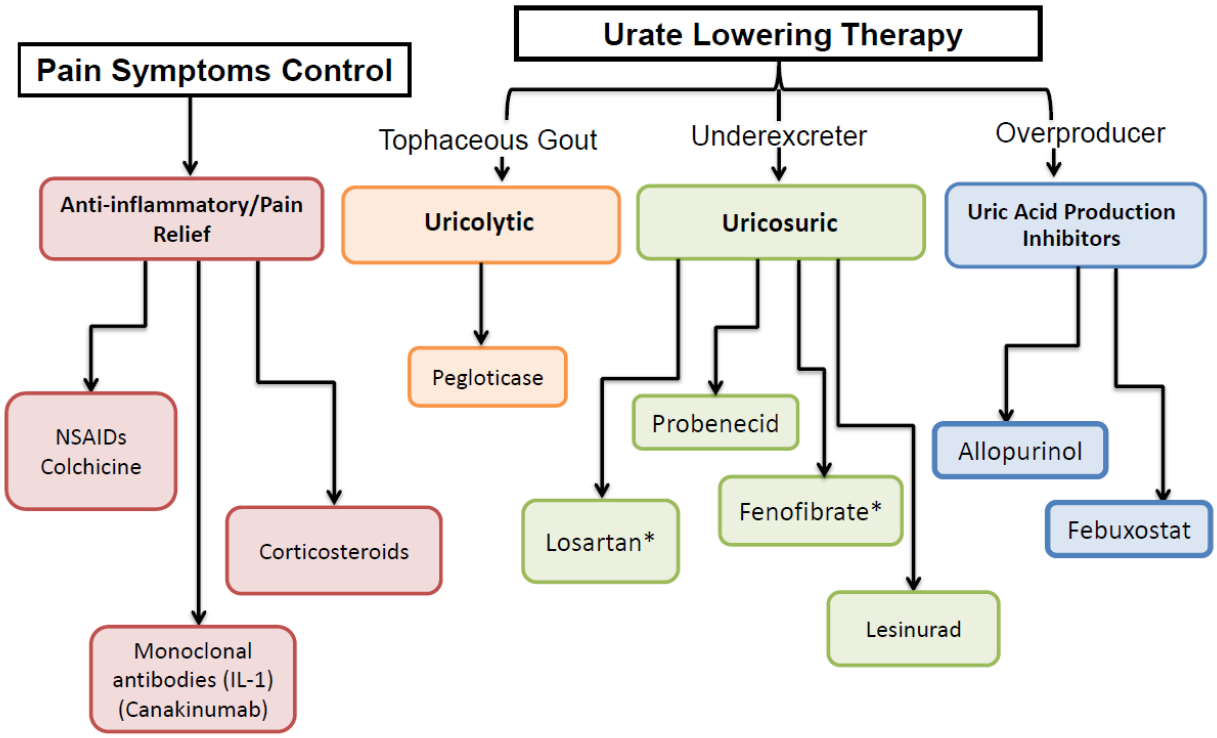


Figure 2:8 Model for hyperuricemia associated comorbidities.
This figure a more mechanism based for the interplay between the hyperuricemia and kidney diseases and kidney associated diseases



NO: Nitric Oxide
RAAS: Renin Angiotensin Aldosterone System
CKD: Chronic Kidney Disease

Figure 2:9 Schematic diagram of the treatment modalities in managing gout. This figure depicts the 2-fold approach of managing gout- control of gout symptoms and targeted urate lowering therapy by gout classification.



* Not FDA Approved for the treatment of hyperuricemia or gout

Table 2:1 Hippocrates' Five Aphorisms on Gout[59]	
Aphorism	**Detail**
VI-28	Eunuchs do not take the gout, not become bald
VI-29	A woman does not take the gout, unless her menses be stopped
VI-30	A youth does not get gout before sexual intercourse
VI-40	In gouty affections, inflammation subsides within 40 days
VI-55	Gouty affections become active in spring and autumn

Table 2:2 Unadjusted and age-adjusted comparison of the prevalence of gout and hyperuricemia among US adults between NHANES-III (1988-1994) and NHANES 2007-2008*[11]			
	NHANES-III 1988-1994	NHANES 2007-2008	Difference
Prevalence of gout (%)			
Unadjusted	2.7 (2.3, 3.0)	3.9 (3.3, 4.4)	1.2 (0.6, 1.9)
Age-adjusted	2.9 (2.5, 3.3)	3.9 (3.4, 4.5)	1.0 (0.4, 1.7)
Prevalence of hyperuricemia (%)	18.2 (17.2, 19.3)	21.4 (19.7, 23.2)	3.2 (1.2, 5.2)
Unadjusted	19.1 (18.1, 20.0)	21.5 (20.1, 23.0)	2.4 (0.7, 4.2)
Age-adjusted			
Mean SUA, mg/dL			
Unadjusted	5.33 (5.29, 5.37)	5.48 (5.41, 5.55)	0.15 (0.07, 0.24)
Age-adjusted	5.36 (5.32, 5.40)	5.49 (5.44, 5.53)	0.13 (0.07, 0.18)

SUA: Serum Uric Acid

* Values are the percent (95% confidence interval). The data were adjusted for clusters and strata of the complex sample design of the National Health and Nutrition Examination Survey (NHANES) 2007-2008, with incorporation of sample weights.

Table 2:3 Relative risk of incident gout among antihypertensive drug classes[95]		
Drug Class	**Relative Risk**	**95% Confidence Interval**
Calcium channel blockers	0.87	0.82 to 0.93
Losartan	0.81	0.70 to 0.94
Diuretics	2.36	2.21 to 2.52
Beta blockers	1.48	1.17 to 1.32
Angiotensin converting enzyme	1.24	1.17 to 1.32
Non-Losartan angiotensin II receptor blockers	1.29	1.16 to 1.43

Table 2:4 Causes, risk factors, and classification of hyperuricemia[69,70]		
Urate overproduction	**Urate underexcretion**	**Combined**
Primary	**Primary**	**Primary**
Idiopathic HGPRT deficiency (Lesch-Nyhan) Keely-Seegmiller syndrome PRPP synthetase over-activity	Idiopathic Familial Juvenile Gouty Nephropathy (FJGN)	
Secondary	**Secondary**	**Secondary**
Purine-rich diet Increased cell turnover Tumor lysis Glycogenoses types III, V, VII	Drugs Renal insufficiency Medullary cystic disease Hypertension Dyslipidemia Acidosis (lactic and keto) Lead poisoning Hypothyroidism Hyperparathyroidism Sarcoidosis Down Syndrome	Alcohol Exercise Shock Glycogenosis type I Aldolase-B deficiency

HGPRT: Hypoxanthine-guanine phosphoribosyltransferase
PRPP: Phosphoribosyl pyrophosphate

Table 2:5 Interpretation of 24-hour urine uric acid before and after a low purine diet[69]			
	Pre-low purine diet (mmol/d) (mg/d)	**Post-low purine diet (mmol/d) (mg/d)**	**Management**
High purine intake	> 6.0 (1000)	< 4.0 (650)	Diet
Overproducers	> 6.0 (1000)	> 4.5 (750)	Family studies Enzyme studies Consider risk of uric acid nephropathy and stones
Underexcreters	< 6.0 (1000)	< 2.0 (350)	Check FEUA

FEUA: Fractional Excretion of Uric Acid

Table 2:6 Uric Acid Handling Classifications[183]

		Urinary Uric Acid Excretion Rate (UUE) (mg/hr/1.73m^2)	
		UUE > 25mg/hr/1.73m^2	UUE $\leq$ 25mg/hr/1.73m^2
% Fractional Excretion of Uric Acid (FEUA%)	FEUA $\geq$ 5.5%	**Uric acid overproduction (ROL)**	**Normal uric acid handling**
	FEUA < 5.5%	**Uric acid overproduction and uric acid under excretion (Combined)**	**Uric acid under excretion (RUE)**

ROL: Renal overload
RUE: Renal underexcretion

Table 2:7 Uric acid normal reference intervals per age group and gender[69]	
Adult Males	0.20- 0.42 mmol/L (3.4 -7.1 mg/dL)
Adult Females	0.14-0.36 mmol/L (2.4-6.1mg/dL)
Children	0.10-0.30 mmol/L (1.7-5.1 mg/dL)

To convert uric acid mg/dL to mmol/L, multiply by 0.05948

Table 2:8 Guidelines for the management of acute gout attacks[149]		
	2016 European League Against Rheumatism Recommendations[8]	**2012 American College of Rheumatology Guidelines for Management of Gout[9]**
First-line agents	Oral colchicine and/or NSAIDs (+PPI if appropriate), CS	Oral colchicine, NSAIDs, or CS
Choice of drug	NSAIDs and colchicine should not be used in patients with severe renal impairment Colchicine should be started within 12 h of flare onset	Choice of drug depends on patient, physician preference, comorbidities, and contraindications Initiation within 12-14 h after attack onset Treatment may need to continue for 7-10 d
Oral colchicine	Loading dose of 1 mg followed 1 h later by 0.5mg Should not be given to patients receiving strong CYP3A4 inhibitors	1.2 mg administered as soon as possible, followed by 0.6 mg 1 h later; followed by 0.6 mg once or twice daily until attack resolves Initiation not recommended ≥ 36 h after attack onset Not recommended when attack in a patient taking colchicine prophylaxis
Intra-articular aspiration and long-acting CS injections	Effective and safe after septic arthritis ruled out	Effective and safe after sufficient precautions have been taken Intra-articular CS favored in attacks involving 1-2 joints
ULT started during an attack	No recommendations	ULT could be started during an attack, if inflammation is controlled Differs from common practice of starting ULT 2-4 wk from resolution of an attack before starting ULT
IL-1 inhibitors		Canakinumab, approved by the EMEA for treatment of adult patients with frequent gout attacks not included in recommendations
Combination treatment	Combination of colchicine and NSAIDs in severe attacks	Combination of colchicine and NSAIDs in severe attacks

Abbreviations: CS, corticosteroids; EMEA, European Medicines Agency; IL-1, interleukin-1; NSAIDs, nonsteroidal anti-inflammatory drugs; ULT, urate-lowering therapy

Table 2:9 Pharmacologic agents for acute gout attacks[145]				
Drug Class	**Drugs**	**Dosing**	**Adverse effects***	**Considerations**
NSAIDS (first-line)	Indomethacin Naproxen Sulindac	Use FDA-approved anti-inflammatory or analgesic doses	GI pain, dyspepsia, heartburn, nausea	Caution required for patients with PUD, active bleeding, anticoagulation or antiplatelet therapy, renal dysfunction, CKD, HTN, fluid retention, CHF, or hepatic disease
Anti-gout agent (first-line)	Colchicine	1.2 mg loading dose and 0.6 mg 1 hr later, then 0.6 mg after 12 hrs, as needed, 1-2 times/d until symptoms resolve	GI (cramping, abdominal pain, nausea, vomiting, diarrhea)	Initiate within 12 hrs after symptom onset. If patient has received colchicine therapy within 14 days, use an NSAID or corticosteroid instead. Caution required for patients with renal dysfunction, CKD, or hepatic disease Contraindicated with concurrent use of P-glycoprotein or CYP3A4 inhibitors
Systemic CS (first-line)	Prednisone	0.5 mg/kg/d for 5-10 days OR 2-5 days at full dose, then taper for 7-10 days	GI (abdominal distension, ulcers), hypertension, headache, insomnia	Caution required for patients with DM, fluid retention, ongoing infection or increased infection risk, or PUD IA, IV or IM administration possible for patients who are NPO
	Methyl-prednisolone	Methylprednisolone dose pack: Taper per package instructions		
Hormone (stimulates CS production)	ACTH	25-40 IU SC	Musculoskeletal, endocrine, metabolic, GI, cardiovascular, nervous system, dermatological effects, hypersensitivity reactions	Options for patients who are NPO. Less effective in patients on long-term oral CS therapy. Short duration of action

ACTH, adrenocorticotropic hormone; CHF, congestive heart failure; CKD, chronic kidney disease; DM, diabetes mellitus; FDA, US food and drug administration, GI, gastrointestinal; HTN, hypertension; IA, intra-articular; IM, intramuscular; IU, international unit; IV, intravenous; NPO, nothing by mouth; NSAID, nonsteroidal anti-inflammatory drug; PUD, peptic ulcer disease; SC, subcutaneous, CS, Corticosteroids * Partial listing

Table 2:10 Summaries of urate-lowering effects of losartan and fenofibrate studies				
Study Drug	**Study design**	**SUA Change**	**Study Comments**	**Reference**
Losartan 50mg QD (n=16) Vs. Candesartan 8mg QD (n=16) for 1 month	Comparative prospective randomized trial in patients with HTN	10% (S) reduction with losartan and no change with candesartan	Japanese cohort	PMID: 18670416
Losartan 50mg QD Vs. enalapril in transplant patients treated with CsA (n=13) for 3 weeks	Prospective, open, randomized, two-way cross-over study	Losartan (NS) reduced SUA 7.8 to 7.3mg/dl, enalapril (NS) increased SUA 7.8 to 8.2mg/dl		PMID: 11328912
Losartan 100mg QD x 2wks in PTs with HTN (n=101)	URAT1 polymorphisms and SUA reduction with losartan	11% (S) reduction with losartan w/up to 23% in rs1529909 (TT)	Chinese cohort rs3825016 is ↑ in hypertensive PTs w/HU	PMID: 26086348
Losartan 50mg QD then BID vs. Irbesartan 150mg QD then BID x 4wks (n=13)	Randomized double-blind controlled cross-over in PTs with HU, Gout, and HTN	Losartan QD (S)↓ SUA from 9 to 8.3mg/dL. Irbesartan had no effect on SUA	European cohort Poor compliance with BID dosing	PMID: 11593107
Losartan 50mg QD + BZB 50 QD (n=13) or ALL 200 BID (n=12)	Prospective, add-on therapy in JPT patients treated for gout and to treat HTN	8% (S)↓ in SUA after 2 months when added to BZB (n=13) or ALL (n=12)	losartan (S)↑ the 24-hr UUE rate and CL_{UA}	PMID: 12759298
Fenofibrate 300mg QD + BZB(n=13) or ALL(n=12)	Prospective, add-on therapy in JPT patients treated for gout and to treat hyperlipidemia	15% (S) ↓ in SUA after 2 months when added to BZB (n=13) and ALL (n=14)	Fenofibrate (S) ↑ the 24-hr UUE rate and CL_{UA}	
Fenofibrate 160 mg daily for 2 months (n=14)	Prospective open label pilot study in Korean gouty patients	23% (S)↓ in SUA after 2 months (6.93-5.22 mg/dL), SCr (S) ↑(1.12-1.27mg/dL)	100% males 43% had dyslipidemia	PMID: 16913436
Fenofibrate 200mg QD (n=10) x 3 weeks	Open label cross-over study with fixed dose of ALL	19% (S) ↓ in SUA after 3 weeks	100% UK male 36% (S)↑ CL_{UA}	PMID: 12595630
Single dose Fenofibrate 300mg (n=9)	Single dose administration in 9 healthy JPT males	26% significant reduction in SUA over 12 hr	Fenofibrate is a URAT1 inhibitor in	PMID: 20075570

QD, daily; BID, twice daily; (S), statistically significant; (NS), non-statistically significant; UUE, Urinary uric acid excretion; HU, hyperuricemia; SUA, serum uric acid; Cl_{UA}, uric acid clearance; ALL, allopurinol, BZB, benzbromarone, CsA, cyclosporine

Table 2:11 Considerations of using urate-lowering therapy[145]			
Class	**Drug**	**Dosing**	**Considerations**
Xanthine oxidase inhibitor (first-line)	Allopurinol	Initiate at 100 mg/d; titrate upward by 100 mg every 2-5 weeks to reach target serum urate acid level; maximum dose: 800 mg/d	Requires renal dosing: Initiate at 50 mg/day for patients with CKD (stage 4 or worse) Potentially fatal hypersensitivity syndrome (testing recommended for high risk groups[†])
	Febuxostat	Initiate at 40 mg/d; titrate to maximum dose (80 mg/d) after 2 weeks if serum uric acid level is not achieved	May cause liver enzyme elevation, arthralgia, or rash Liver tests needed in patients who develop fatigue, anorexia, right upper abdominal discomfort, dark urine, or jaundice
Uricosuric Agent (Second-line)	Probenecid	250 mg twice daily for 1 week, then 500 mg twice daily; titrate in 500 mg increments every 4 weeks until target serum uric acid level is reached; maximum dose: 2 g/day	Avoid in patients with history of urolithiasis Caution in eCrCL <50 mL/min
	Lesinurad	200mg daily in combination with XOI	Avoid use in patients with eCrCl <45ml/min May cause acute renal failure if used as a monotherapy
	Losartan** (ARB)	No FDA-approved dosing	Useful for patients with hypertension
	Fenofibrate**	No FDA-approved dosing	Useful for patients with dyslipidemia
Uricolytic	Pegloticase	8 mg IV every 2 weeks	IV infusion over ≥120 min Severe infusion and allergic reactions possible May exacerbate HF Contraindicated in patients with G6PD deficiently High cost

ARB, angiotensin receptor blocker; HF, heart failure; CKD, chronic kidney disease; FDA, US Food and Drug Administration; HTN, hypertension; IV, intravenous; G6PD, Glucose-6-Phosphate Dehydrogenase, XOI, Xanthine Oxidase Inhibitor. * Patients receiving urate-lowering therapy should be started on anti-inflammatory agent, as well. † Koreans with CKD (stage 3 or worse) and all Han-Chinese and Thai patients
**Not FDA Approved for the management of gout or hyperuricemia

Table 2:12 Dietary recommendations for patients with gout[145]		
Avoid	**Limit**	**Encourage**
Organ meat high in purine content (sweetbreads, liver, kidney)	Serving sizes of purine-rich meats (beef, lamb, pork) and seafood (sardines, shellfish)	Low-fat or nonfat dairy products
High fructose corn syrup-sweetened sodas and other beverages and foods	Serving sizes of fruit juice, table sugar, sweetened beverages and desserts, and table salt, including salt in sauces and gravies	Vegetables
Alcohol overuse (>2 drinks/day for men and >1 drink/day for women). Refrain from all alcohol during periods of frequent gout attacks or advanced, poorly controlled gout	Alcohol (particularly beer, but also wine and spirits)	

Chapter 3

Assessment of Genetic Polymorphisms Associated with Hyperuricemia or Gout in the Hmong

Introduction:

The global prevalence of hyperuricemia has grown dramatically over the past two decades.[15] In the United States, the prevalence of hyperuricemia significantly increased from 18.2% in 1988-1994 to 21.4% in 2007-2008[11,15,18]. Hyperuricemia is typically classified by serum uric acid (SUA) $\geq$ 6.0mg/dL and is the strongest predictor of gout, the most common inflammatory arthropathy disease [134,143,184]. When serum uric acid levels exceeds its solubility threshold ($\geq$ 6.8mg/dL), the risk of monosodium urate crystal formation is high and often leads to a variety of acute and chronic complications. Although fundamentally a metabolic disorder, hyperuricemia has also been strongly associated with increased risk for cardiovascular diseases, chronic kidney disease, renal stones, and new onset of type 2 diabetes mellitus, which all have a significant impact on morbidity and mortality[56,185,186].

Hyperuricemia results from an imbalance between the production and excretion of SUA. Overproduction of uric acid (UA) may be attributed to diets rich in purine content [141] or pathological conditions leading to accelerated breakdown or production of endogenous nucleic acids. While breakdown of endogenous purines represents the main sources of SUA, underexcretion of UA is typically the most common cause of hyperuricemia[21]. Uric acid is primarily excreted renally with only 30% being eliminated non-renally. Specifically, UA excretion takes place in the proximal convoluted tubule (PCT) yet, the majority ($\sim$ 90%) of filtered UA is reabsorbed in the descending renal tubule[21]. Uric acid excretion is confounded by declining kidney function, concomitant use of select drugs (e.g. niacin, low dose aspirin, thiazides, and loop diuretics) and urine pH.

Several non-modifiable factors such as age, gender, and race can also influence SUA levels.

Considerable variability in the prevalence of hyperuricemia and gout across various races and ethnicities has been described.[78] For example, African-Americans,[82] Maori,[9] Japanese,[12] and Hmong,[17] have each been shown to have a higher prevalence of hyperuricemia or gout relative to Caucasians, raising the suspicion that there is a genetic basis for this observation.[9] These observations are supported by studies which have identified a differential prevalence of single nucleotide polymorphisms (SNPs) within genes affecting the urate transportome,[80-82] which is in part responsible for elevated SUA levels.[187-191] Genome wide association studies (GWAS) have identified transporters including SLC22A12, SLC2A9, ABCG2, and *SLC17A1* as well as the scaffolding protein PDZK1, to have the highest degree of impact on SUA.[192] Although other genes are involved, their mechanisms of contribution to elevated SUA remain unclear.[193,194]

Minnesota Hmong represent an important, underserved minority group numbering over 64,000.[195] Hmong patients suffer from a 2-fold higher prevalence of gout [26] and 5-fold higher incidence of UA ureteral and kidney stones,[17,24] compared to non-Hmong Minnesotan patients. To ascertain if genetic polymorphisms may contribute to the Hmong's predisposition to hyperuricemia and gout, the frequencies of validated risk alleles within five candidate genes *(SLC22A22, SLC2A9, ABCG2, SLC17A1, and PDZK1)* were quantified in a Hmong cohort, and compared with published data from a racially concordant population of Han-Chinese in Beijing (CHB) and a racially discordant population of Utah residents with ancestry from northern and western Europe (CEU). In

addition, the association between baseline SUA concentration and risk alleles was explored within a subset of the Hmong cohort.

Methods:

Study Participants

Using the principles of community-based participatory research,[196] we created and partnered with the Hmong Genomics Board, which co-designed the study, recruited the participants, and collected the data. From March 2009 through May 2009, self-reported Hmong adults ≥ 18 years of age were recruited in various settings including; medical clinics, three college campuses, a Minnesota Hmong college student conference and a Hmong national conference held in Wisconsin. As part of the consent process, people received written and verbal information in Hmong or English about what genes are, how they vary in populations, and why they might affect health and medication use. Once informed consent was obtained, saliva samples were collected using ORAgene® OGR-500 kits (Ottawa, ON, Canada) per manufacturer's protocol. In addition, demographic information (age, gender, years in US, formal education), medical history (past medical history, medicines, and family history) and anthropometric measurements (heights, weights, waist circumference and blood pressure) were collected. Additionally, although not the primary purpose of this study, we offered to perform blood tests (specifically SUA) on participants ≥ 30 years of age and based on their willingness to provide blood samples. This project was approved by the University of Minnesota Human Research Protection Program (UMN IRB # 0711M21884).

SNP Selection Criteria

Three primary GWAS papers investigating the association of SNPs with SUA were used to guide our approach to selecting the primary genes and relevant SNPs. The first was a GWAS by *Vitari et al* [197] which included 986 Croatian's isolate and identified SLC2A9 to be associated with SUA levels, fractional excretion of uric acid (FEUA) and gout. The second was a meta-analysis by *Kolz et al* [80]which included 28,141 subjects of European descent and identified 9 genes and 9 loci to be associated with SUA levels. The third study was another meta-analysis by *Kottgen et al,*[81] consisting of over 140,000 subjects from a European ancestry. *Kottgen et al* [81] identified 18 new loci in association with SUA using Gene Relationships Across Implicated Loci approach. The latter meta-analysis is significant for two reasons. First, it provided validation of previously identified SNPs associated with elevated SUA and augmented that data with another phenotype – namely FEUA. Further, it specifically evaluated whether the observed SNP associations were generalizable to individuals of non-European ancestry. By replicating their findings within large cohorts of African-Americans, Indians and Japanese populations, it concluded that the SNP effects on SUA concentrations were comparable in magnitude and identical in direction for the majority of SNPs studied [81]. Therefore, key SNPs identified by any of these studies were considered important to pursue in addition to other SNPs identified by candidate gene approaches using phenotypes such as FEUA, fructose load test and simply the presence of gout [198,199].

The final SNP list for the present study was further filtered by considering only those SNPs in genes directly associated with UA homeostasis with a reported minor allele frequencies in excess of 5% based on data from CEU[200] and not otherwise in linkage

disequilibrium ($r^2 \leq 0.8$) with other SNPs. Our final list included 8 SNPs in 5 genes directly associated with SUA homeostasis.

Genotyping

DNA was purified and extracted from 235 saliva samples. Briefly, 4 mL of saliva was incubated in a water bath at 50°C overnight. Following incubation, the appropriate amount of purifier was added followed by 10 minutes of ice incubation. The samples were centrifuged for 20 minutes at maximum speed (4000rpm) at room temperature. In order to precipitate the DNA, the yielded supernatant after centrifugation was transferred to new centrifuge tubes followed by adding an equivalent amount of ethanol and then kept in the refrigerator overnight. Samples were then centrifuged for 20 minutes at maximum speed (4000 rpm) at room temperature. The yielded supernatant was then discarded and the DNA pellet was dried for 30 minutes followed by adding 1 mL of Tris-EDTA (TE) buffer placed on a rotator (34rpm) overnight. Finally, samples were prepared, labeled, and analyzed for quality and quantity using an eight-multichannel Nano-drop (ND8000 V.2.). Eight candidate SNPs in five key genes were genotyped at the Biomedical Genomic Center (BMCG) at the University of Minnesota (Minneapolis, MN, USA). Six SNPs were genotyped by Sequenome iPLEX Gold method using Bruker Autoflex II MALDI/TOF mass spectrometer (rs505802, rs2231142, rs12129861, rs11942223, rs1014290, rs1183201). The other two SNPs (rs3733591, rs734553) were genotyped by TaqMan® applied biosystems™.

Sequenom Genotyping

Genotyping was performed using iPLEX Gold method. iPLEX reagents and protocols for multiplex PCR, single base primer extension (SBE) and generation of mass

spectra, as per the manufacturer's instructions. Multiplexed PCR was performed in 5-µl reactions on 96-well plates containing 10 ng of genomic DNA. Reactions contain 0.5 U HotStar Taq polymerase (QIAGEN), 100 nM primers, 1.25X HotStar Taq buffer, 1.625 mM MgCl2, and 500 µM dNTPs. Following enzyme activation at 94 °C for 15 min, DNA was amplified with 45 cycles of 94 °C x 20 secs, 56 °C x 30 secs, 72 °C x 1 min, followed by a 3-min extension at 72 °C. Unincorporated dNTPs were removed using shrimp alkaline phosphatase (0.3 U, Sequenom). Single-base extension was carried out by addition of SBE primers at concentrations from 0.625 µM (low MW primers) to 1.25 µM (high MW primers) using iPLEX enzyme and buffers (Sequenom, San Diego, CA, USA) in 9-µl reactions. Reactions were desalted and SBE products measured using the MassARRAY system, and mass spectra analyzed using TYPER software (Sequenom, San Diego, CA, USA), in order to generate genotype calls and allele frequencies.

Statistical Analysis

Data from the HapMap[200] served as our source for the frequencies of the variant alleles in CEU and CHB populations. The allele frequencies were then compared to Hmong allele frequencies using Pearson's Chi-Square or Fisher's exact test when appropriate based on cell count. A Bonferroni adjustment was used to account for multiple comparisons between *risk alleles* using a critical value $p<0.0063$ for significance. In our analysis, the "*risk allele*" was defined as the allele that was associated with higher SUA or could adversely increase SUA levels. Using the American College of Rheumatology Guidelines of [SUA] < 6mg/dL in patients treated for gout, we defined the *risk* of hyperuricemia in our Hmong cohort as [SUA] $\geq$ 6mg/dL. Ascertaining the association of the risk of hyperuricemia using the SUA cutoff with genotype was calculated using

Pearson's Chi-Square test or Fisher's exact test. Means of SUA were compared across genotypes using One-Way ANOVA.

Results:

Two hundred and thirty-five Hmong adults participated in the study (Figure 3.1). Demographics and anthropometric measures are summarized in Table 3.1. Briefly, the mean (±SD) age of all study participants was 30.3 (±15.7) years with a slight majority of females (55%). More than 60% of the participants had body mass index ≥ 25 kg/m^2. Prevalence of self-reported gout was 5.1%. In addition, 57 participants agreed to have their SUA levels measured, with a mean (±SD) SUA level of 6.3 (± 1.7) mg/dL. Of the 57 participants, 27 (47%) had SUA levels ≥ 6mg/dL.

All the selected SNPs were in Hardy Weinberg equilibrium with call rates >99.0%. Seven of the 8 targeted SNPs were significantly different when comparing the prevalence between Hmong and CEU (Table 3.2-3). Among the 7 SNPs showing a difference, 6 of the risk alleles [rs505802 (C>T), rs2231142 (G>T), rs12129861 (G>A), rs11942223 (T>C), rs734553 (G>T), and rs1183201 (T>A)] were significantly more prevalent in the Hmong compared to the CEU population. However, the prevalence of the risk allele rs3733591 (A>G) was found to be significantly lower in the Hmong compared to CEU. For the comparisons between Hmong and CHB populations, 3/8 SNPs showed statistically significant differences. Two of the risk alleles were significantly more prevalent in the Hmong compared to CHB [rs3733591 (A>G) and rs1014290 (C>T)], and 1 risk allele rs12129861 (G>A) had lower prevalence. For the 57 adults with measured SUA levels, no

significant associations between risk alleles and SUA levels or risk of hyperuricemia (SUA $\geq$ 6mg/dL) were found. Association data analyses are not shown.

Discussion:

This study identifies a statistically higher prevalence of validated risk alleles in our Hmong cohort compared to published data in European (CEU) and Han Chinese (CHB) populations. Specifically, 6/8 risk alleles were more prevalent in the Hmong than CEU, and 2/3 risk alleles were more prevalent in the Hmong than the CHB. This observation represents the first report of its kind, which may support a genetic basis for the notably higher prevalence of hyperuricemia and gout observed in the Hmong, relative to non-Hmong residing in Minnesota. Our findings of differences between Hmong and CHB population further support the importance of utilizing the more precise genomic-based guidance for risk associations rather than race or ethnic classifications. The roles that select genetic variations within genes (*SLC22A12, SLC2A9, ABCG2, SLC17A1 and PDZK1)* affecting UA disposition play in the manifestation of hyperuricemia, development of gout and modulating drug responsiveness are worthy of discussion.

SLC22A12 encodes for the URAT1 transporter found on the apical side of the PCT in kidney. Loss-of-function mutations of URAT1 cause idiopathic renal hypouricemia due to high urinary urate excretion[177] and presumably a gain of function mutation could lead to hyperuricemia[201]. Considered a major carrier facilitating UA reabsorption, this transporter was deemed a logical target for urate lowering therapies. The inter-genic SNP rs505802 (C>T) with an estimated prevalence of the (T) allele at 73% was identified by *Kolz* et al [80] to be associated with a lower SUA. In contrast, the prevalence of the (T) allele in our

Hmong cohort was only 35% ($p < 0.001$) suggesting a higher risk for hyperuricemia in the Hmong, relative to Europeans. Incidentally, the estimated prevalence of the (T) allele in the CHB population was 26% which, although not significantly ($p=0.0068$) lower compared to our Hmong cohort, suggests that the Hmong and CHB populations (both considered to be Asian) appear to display substantive differences in allele frequencies. Two other SNPs [rs893006 (A>G), and rs11231825 (C>T)] within *SLC22A12* were found to be significantly associated with elevated levels of SUA in a Japanese cohort, [188] or reduced fractional excretion of uric acid (FEUA) in a German population.[187] Notably, both of these SNPs are in complete linkage disequilibrium (LD, $r^2=1$) with the previously mentioned rs505802.

Consequently, not only does rs505802 influence renal UA disposition, but it may also influence response to urate lowering therapies. The basis for this argument resides in our understanding of the mechanism of action of two common urate-lowering therapies: probenecid and allopurinol. Both probenecid and more precisely, the active metabolite of allopurinol, known as oxipurinol, inhibit URAT1 by competing for reabsorption with UA.[169,170] Thus, our finding of a higher prevalence of the risk allele associated with hyperuricemia (rs505802C>T) may not only partially explain the higher prevalence of gout in the Hmong, but also provide a basis for anecdotal reports questioning the effectiveness of allopurinol when used in the Hmong [26]. Genotype based differences on renal handling of UA and the effectiveness of urate lowering therapies complicate the interpretation of risk for hyperuricemia and response to select medications. Studies that

de-couple these competing factors should elucidate our understanding of the potential basis for genotype-guided selection of drug therapy.

SLC2A9 encodes for GLUT9 transporter which is a high capacity transporter for fructose, glucose, and UA. GLUT9 is not only expressed in the kidney, liver, but it is also expressed in the chondrocyte of human articular cartilage.[202] The latter maybe an important contributing factor for the deposition of monosodium urate crystals within and around joints affected with gout. The intronic SNP rs734553 (G>T), was shown to be significantly associated with increased SUA in the CEU population with an estimated prevalence of the (T) allele at 74%.[80] In our Hmong cohort, the estimated prevalence of the (T) allele was 99%; which was significantly higher than CEU (P<0.0001). In this case, the prevalence of the (T) allele was not different between the Hmong and CHB, both at ~99% (p=0.269). Once again, the Hmong's genetic predisposition to hyperuricemia is enhanced relative to CEU. Notably, the SNP rs734553 (G>T), is in moderate LD (r^2= 0.8) with the intronic SNP rs11942223 (T>C) for which the (C) allele has been shown to be protective against hyperuricemia.[198] The prevalence of the protective (C) allele in our Hmong cohort (1.3%), was again lower prevalence compared to CEU (25%) (p<0.001).

Also within *SLC2A9*, the non-synonymous SNP rs3733591 (Arg265His) has been consistently associated with gout. For instance, *Tu et al* [199] identified that a copy of the risk allele (C) had a higher risk for tophi with an OR of 2.05 (1.11-3.77, p=0.0176) in the Han-Chinese population while *Urano et al*[203] demonstrated an increased risk for gout in Japanese with an OR of 1.52 (1.19-1.95, $p=7.3x10^{-4}$). In our Hmong cohort, the risk allele rs3733591 (A>G) is lower (41%) compared to CEU (80%) (p<0.001), but higher than

CHB (30.7%) (p=0.005) further suggesting that the Hmong is a unique ethnic group in spite of being commonly classified as CHB.

Reduced FEUA is common in patients with gout and is a risk factor for developing hyperuricemia. The intronic SNP rs1014290 (C>T) within SLC2A9 identified by *Vitart et al* [197] was found to be significantly associated with decreased FEUA and development of gout in multiple populations of European descent. The presence of the T allele was significantly associated with OR (1.57 and 1.40) for reduced FEUA and gout, respectively. In our present study, the prevalence of T allele in our Hmong population (69.5%) was lower than CEU (74.3%, p=0.19), but higher than in CHB (59.6%, p=0.006). This further suggests that decreased FEUA may be a contributing mechanism for developing gout in the Hmong which, again, differs from the CHB.

SLC17A1 encodes the voltage gated human sodium-dependent phosphate co-transporter type 1 protein (NPT1) on the apical membrane of the renal proximal tubule. *Chiba et al* [204]have recently identified the putative mechanism and role by which NPT1 affects uric acid suggesting that NPT1 functions as a renal urate excreter. In a Japanese case-control study these same investigators also identified the missense SNP rs1165196 (I269T) of *SLC17A1* gene to be a gain of function change which could protect against renal urate underexcretion. *Kolz et al* [80]identified the intronic SNP rs1183201 (T>A) of the *SLC17A1* gene to be significantly associated with decreased serum uric acid with the effect allele (A) being the protective allele in CEU. In addition, this SNP was also found within a larger region on chromosome 6 suggesting its high LD with other SNPs. This finding could explain the moderate LD (r^2 =0.868) between these two SNPs (rs1165196

and rs1183201) in CEU and strong LD (0.967) within CHB. *Zhou et al* [205]have successfully replicated similar findings in CHB males case-control study by showing that SNP rs1183201 was significantly associated with gout and SUA and in consistency with *Kolz et al* findings.[80]

In our Hmong cohort, the frequency of the effect allele (A) for rs1183201 was 3-fold lower than that observed in Caucasians (16.4% vs. 50%, p<0.001). However, the frequency of the effect allele did not differ between Hmong and CHB (16.4% vs. 13.3%, p=0.54). The extensive validation of this SNP (rs1183201) across different ethnic groups and its direct role on renal uric acid excretion highlights a plausible mechanism for the Hmong to be at higher risk for developing gout, relative to other populations. These consistent findings in part, provide possible genetic basis for the differential prevalence of gout in Hmong relative to non-Hmong living in Minnesota[17].

ABCG2 encodes for the superfamily of ATP-binding cassette (ABC) protein which is a xenobiotic efflux transporter and major carrier for endogenous substances. Genetic polymorphisms in *ABCG2* have been associated with SUA and gout in select populations.[206-208] The missense SNP (rs2231142) Gln141Lys (G>T) has been shown to be associated with elevated SUA in CEU men [80] and indeed, the prevalence of the risk allele (T) in our Hmong cohort (36%) was higher than that in the CEU population (11%) (p<0.001). Significantly, this SNP (rs2231142) has also been strongly associated with response to allopurinol in White non-Hispanic and Hispanic populations.[86] This observation further strengthens the argument that genetic variability of transporters is associated with disposition of UA and could also modulate drug response.

The scaffolding structural protein PDZK1's role on UA disposition was evident from the meta-analysis conducted by *Kolz et al* [80] who showed that the inter-genic SNP rs12129861 (G>A) was significantly associated with lower SUA levels. This protein is essential for UA transporter activity including URAT1, OAT4, and NPT1.[182] The prevalence of the (A) allele for rs12129861 (G>A), associated with decreased SUA, in our Hmong cohort (33%) was lower than CEU (46%) ($p < 0.001$), but was higher than CHB (20%, $p=0.002$). This finding is consistent with the elevated risk of gout in the Hmong relative to CEU while being supportive of our premise that there are differences between Hmong and CHB.

Finally, while different allele frequencies in Hmong could owe to their unique population history rather than a genuine causal effect on hyperuricemia or gout, we believe that the extensive validation of these selected SNPs across different population groups and ethnicities provide the basis to theorize that the different allele frequencies in the Hmong may be contributing to their enhanced risk of gout compared to CEU.

In general, advancing age increases the risk of developing gout due to several factors including declining kidney function, increased dehydration, increased use of drugs and increased prevalence of other comorbidities. The estimated prevalence of gout in our Hmong cohort was 5.1% which is close to the 6.2% reported by Portis et al[17] and notably higher than the overall US prevalence of 3.9%.[11] Our findings may provide evidence to support a genetic component to the clinical observations of a higher prevalence of gout, hyperuricemia, and UA kidney stones[17,24] and reported suboptimal use of allopurinol[26] in Minnesota Hmong compared to non-Hmong. The consistency of urate transportome risk

alleles within our Hmong cohort compared to other populations is at the very least suggestive of a genetic component to their elevated risk. However, several limitations of our study require further discussion.

Limitations:

The key limitations of our study include those common to investigations of complex questions within minority populations. In our example, the Hmong are a minority refugee population in which to conduct research, especially involving the collection of DNA or blood is challenging. However, working with the Hmong Genomics Board and translators as partners in research, ensured that participants were appropriately informed as to the nature and purpose of the study. Another limitation of our study relates to sample size. Although the total sample size (n=235) represents a reasonable basis to estimate the prevalence of the 8 targeted SNPs, the modest sample size of individuals with measured SUA (n=57) and gout (n=12), limits the power of our analysis to determine an association between the genotypes tested, and risk of hyperuricemia or gout. Similarly, our sample size precluded meaningful analyses of these associations based on a sex-specific definition of hyperuricemia that otherwise may be more appropriate than our clinically oriented cut off for hyperuricemia of SUA $\geq$ 6mg/dL. We cannot exclude the possibility of a selection bias in that those who volunteered to have their SUA measured may differ from those who did not volunteer. A larger and appropriately powered study will be needed to ascertain the effect of these select SNPs on SUA levels and the presence of gout.

Although some investigators have found an association with SUA and gout with other genes (*GCKR* and *LRRC16A*),[80,205,209] these genes were not evaluated in our

population. Further investigations of direct associations with these genes and key other SNPs are worthy of direct study in this unique population. Finally, we did not have comprehensive information for concomitant use of drugs or dietary habits, both of which may have influenced SUA levels. Indeed, diet is an important determinant of SUA levels and is cited as a key common risk factor in those populations exhibiting elevated prevalence of gout and hyperuricemia.[31] Any additional study will need to explore dietary variations and its association with gout

Conclusions:

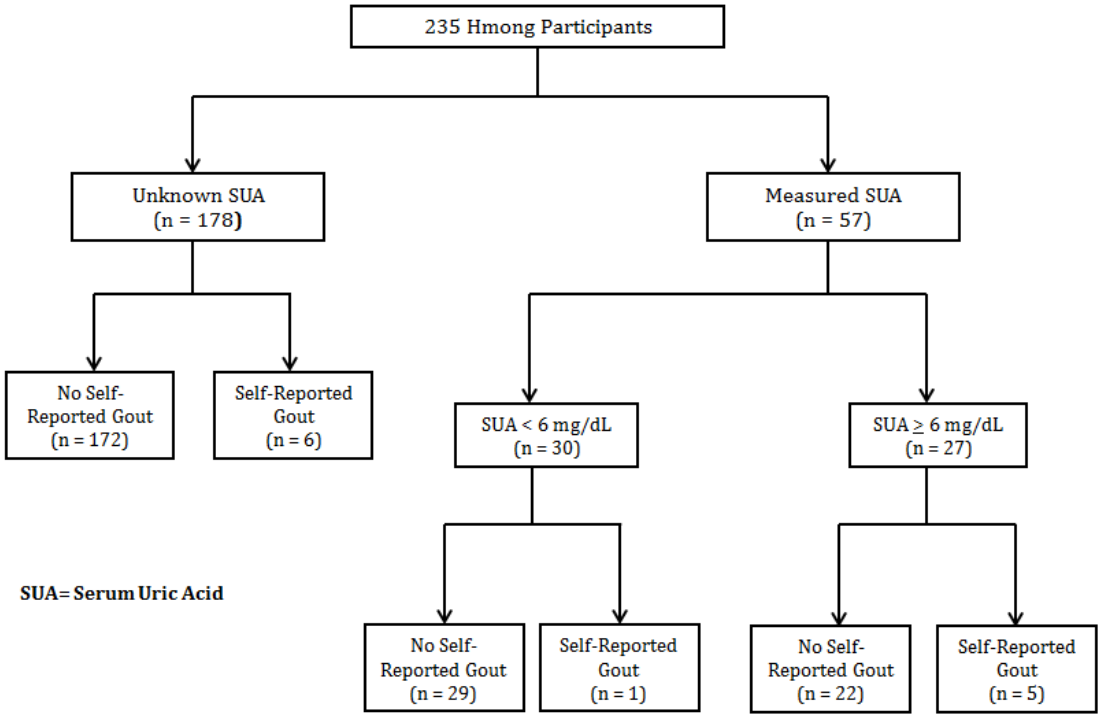
Hyperuricemia risk alleles are more prevalent in the Hmong than CEU and CHB and are directionally consistent with the increased risk of hyperuricemia and gout when compared with either CEU or CHB populations. Although the mean age in our Hmong cohort was significantly lower relative to previous reports and we had a preponderance of female subjects, the Hmong in this study remain to exhibit a higher prevalence of self-reported gout compared to the US population. Given the extensive work by others in replicating the role of key SNPs discovered in Europeans to be comparable in magnitude and identical in direction in Indians, African-Americans and Japanese, we believe that our findings have advanced our knowledge of the risk for hyperuricemia and gout in the Hmong.

Future perspectives:

If replicated, the translational implications of our findings may lead to genomic-based differentiation of prevention and treatment strategies for individuals with specific genetic profiles. The consideration of genetic factors in the selection of therapies may

also lead to improved patient outcomes and lower health care costs. Comparative efficacy studies utilizing genetic information have the potential to improve pharmacotherapeutic outcomes by avoiding un-necessary trials of less efficacious drug therapies. Ultimately, this genetic information may advance our capacity to apply principles of precision medicine to achieve improved outcomes. In addition, individual's genotype may ultimately replace the imprecise descriptors such as race when considering individual's risk for gout and/or response to gout therapy.

Figure 3:1Risk of Hyperuricemia* and Self-reported Gout in 235 Participants



Distribution of individuals among those with unknown or measured serum uric acid (SUA) by self-reported history of gout. *Risk for hyperuricemia in our study was defined as SUA ≥ 6mg/dL

Table 3:1 Clinical characteristics and demographics of study participants (n=235)			
Participant Characteristics	**No. (%)or Mean ±SD**	**Min**	**Max**
Age (years)	30.3 ± 15.7	18	87
Sex			
Females	130 (55.3)		
Males	105 (44.7)		
Smoking Status			
Never smoked	191 (81.3)		
Former smoker	20 (8.5)		
Current smoker	20 (7.2)		
Missing data	7 (3.0)		
Medical History			
Type 2 Diabetes	31 (13.2)		
Hypertension	15 (6.4)		
Hyperlipidemia	14 (6.0)		
Gout	12 (5.1)		
Kidney Stone	6 (2.6)		
BMI (kg/m^2)	27.8 ± 6.2	16.7	52.1
Serum uric acid (SUA) (mg/dL) (n=57)	6.3 ± 1.7	3.5	11.4
Females SUA (mg/dL) (n=39)	5.9 ± 1.6	3.5	9.9
Males SUA (mg/dL) (n=18)	7.1 ± 1.6	4.8	11.4
Waist Circumference inches/(cm)			
Females	33.3 ± 5.7/ (84.5 ± 14.4)	24.5/ (62)	50.0/ (127)
Males	35.6 ± 5.4 / (90.4 ± 13.6)	26.0/ (66)	51.5/ (131)
Systolic Blood Pressure (mmHg)	117 ± 16	80	178
Diastolic Blood Pressure (mmHg)	72 ± 10	40	101
Self-reported years in USA (years)	15.6 ± 8.7	3	31

Table 3:2 Allele Frequencies within Hmong, European (CEU), and Han-Chinese (CHB)					
Gene (SNP)/Allele*	**Hmong No. (%)**	**CEU No. (%)**	**P. Value Hmong Vs. CEU**	**CHB No. (%)**	**P. Value Hmong Vs. CHB**
SLC22A12 (rs505802)					
C	297 (64.8)	62 (27.4)	**<0.001**	204 (74.5)	0.0068
T	161 (35.2)	164 (72.6)		70 (25.5)	
Total	458	226		274	
SLC2A9 (rs3733591)					
A	265 (58.6)	43 (19.7)	**<0.001**	190 (69.3)	**0.005**
G	187 (41.4)	175 (80.3)		84 (30.7)	
Total	452	218		274	
SLC2A9 (rs11942223)					
T	452 (98.7)	180 (75.0)	**<0.001**	178 (98.9)	1
C	6 (1.3)	60 (25.0)		2 (1.1)	
Total	458	240		180	
SLC2A9 (rs734553)					
T	459 (98.5)	167 (73.9)	**<0.001**	173 (99.6)	0.269
G	7 (15.0)	59 (26.1)		1 (0.4)	
Total	466	226		174	
SLC2A9 (rs1014290)					
T	317 (69.5)	168 (74.3)	0.191	161 (59.6)	**0.006**
C	139 (30.5)	58 (25.7)		109 (40.4)	
Total	456	226		270	
ABCG2 (rs2231142)					
G	293 (63.7)	201 (88.9)	**<0.001**	194 (70.8)	0.059
T	167 (36.3)	25 (11.1)		80 (29.2)	
Total	460	226		274	
SLC17A1(rs1183201)					
A	75 (16.4)	63 (50)	**<0.001**	12 (13.3)	0.559
T	381 (83.6)	63 (50)		78 (86.7)	
Total	456	126		90	
PDZK1 (rs12129861)					
G	307 (67.3)	122 (53.9)	**<0.001**	220 (80.3)	**0.002**
A	149 (32.7)	104 (46.1)		54 (19.7)	
Total	456	226		274	

*Bolded allele indicates the risk allele, which is defined as the allele that is associated with higher SUA or could adversely increase SUA levels)

P<0.0063 indicates statistical significance

Table 3:3 Genotype Frequencies Comparisons between Hmong, European (CEU) and Han-Chinese (CHB)					
Gene (SNP) / Genotype	Hmong No. (%)	CEU No. (%)	P. Value Hmong Vs. CEU	CHB No. (%)	P. Value Hmong Vs. CHB
SLC22A12 (rs505802)					
CC	101 (44)	10(9)		76(55.5)	
CT	95 (41.5)	42(37)	**<0.001**	52(38)	**<0.001**
TT	33(14.5)	61(54)		9(6.6)	
Total	229	112		137	
SLC2A9 (rs1014290)					
TT	105(46.0)	6 (5.3)		23 (17.0)	
TC	107(46.9)	46 (40.7)	**<0.001**	63 (46.7)	**<0.001**
CC	16 (7.0)	61 (54.0)		49 (36.3)	
Total	228	113		135	
SLC2A9 (rs11942223)					
TT	223 (97.4)	33 (50.8)		44 (97.8)	
TC	6 (2.6)	31 (47.7)	**<0.001**	1 (2.2)	0.999
CC	0 (0.0)	1 (1.5)		0 (0)	
Total	229	65		45	
SLC2A9 (rs734553)					
TT	226 (97.0)	60 (53.1)		136 (99.3)	
GT	7 (3.0)	47 (41.6)	<**0.001**	1 (0.7)	0.266
GG	0	6 (5.3)		0	
Total	233	113		137	
SLC2A9 (rs3733591)					
GG	38 (17)	71(65)	**<0.001**	9(7)	0.0077
GA	111(49)	33(30)		66(48)	
AA	77(34)	5(5)		62(45)	
Total	226	109		137	
ABCG2 (rs2231142)					
GG	96 (41.7)	89 (78.8)	**<0.001**	69 (50.4)	0.211
GT	101 (43.9)	23 (20.4)		56 (40.9)	
TT	33 (14.3)	1 (0.9)		12 (8.8)	
Total	230	113		137	

Table 3.3 Cont.					
SLC17A1(rs1183201)					
AA	7 (3.1)	17 (27)	**<0.001**	0	0.489
AT	61 (26.8)	29 (46)		12 (26.7)	
TT	160 (70.2)	17 (27)		33 (73.3)	
Total	228	63		45	
PDZK1 (rs12129861)					
GG	105 (46.1)	35 (31.0)	**0.0039**	88 (64.2)	<0.001
AG	97 (42.5)	52 (46.0)		44 (32.1)	
AA	26 (11.4)	26 (23.0)		5 (3.6)	
Total	228	113		137	

P<0.0063 indicates statistical significance

Chapter 4

Results and Discussion Addendum

Addendum Overview

The following is a summary of select preliminary investigations of allele frequencies for SNPs of very important pharmacogenes, which are relevant to select drug therapies. In addition, we expand our analyses that were previously published in chapter III. The purpose is to summarize likely areas of further investigations when exploring unique genetic differences in disease risk and drug response that may be relevant to the Hmong.

Assessment of Select Pharmacogenes in the Hmong:

The solute carrier organic anion 1B1 gene (*SLCO1B1*) is located on chromosome 12 (p12.1) and encodes the organic anion transporter 1B1 (OAT1B1), which is primarily expressed on the basolateral side of the hepatocyte.[210] The primary function of OAT1B1 transporter is the uptake of endogenous compounds such as bilirubin or xenobiotics such as simvastatin and simvastatin acid from the portal vein into the liver for metabolism. The single nucleotide polymorphism (SNP) rs4149056 T>C (Val174Ala) within the *SLCO1B1* has been identified from a genome-wide association scan (GWAS) to be linked with statin-induced myopathy (SIM).[211] Multiple retrospective studies have validated the association between SIM and the rs4149056 T>C within *SLCO1B1*.[212,213] In fact, patients who are carriers of the risk allele (C) are at a significantly higher risk of developing SIM with more than 4-folds higher than the T allele, which warrants dose reduction or using an alternative to simvastatin.[214] Based on our preliminary analyses of 235 Hmong participants, the prevalence of the risk allele (C) in the Hmong is significantly lower than Europeans (2.6 vs 12.1%, $p<0.001$) (Table 4.1). This may suggest that the Hmong are less likely to develop SIM based on the genetic polymorphism in *SLCO1B1* compared to

European counterparts at least via this mechanism.

The phase (I) metabolism enzyme encoding gene, *CYP2C19* is located on chromosome 10 (q23.33).[210] CYP1C19 is an important metabolizing enzyme for commonly and chronically used drugs such as losartan, phenytoin, citalopram, escitalopram, omeprazole, esomeprazole, and clopidogrel.[215] Specifically, the antiplatelet drug clopidogrel, which is an adenosine diphosphate receptor antagonist, is commonly used in post-myocardial infarction, stroke, peripheral arterial disease patients as well as in patients undergoing percutaneous coronary intervention. Clopidogrel is a prodrug that can be converted into the inactive metabolite (85%) via the carboxylesterase pathway or the active metabolite (15%) via CYP2C19 activity. The response to clopidogrel is highly variable and is considered to be infective in up to 30% of patients taking it.[216] Genetic variations in *CYP2C19* have been strongly associated with the response to clopidogrel leading to the development of genetic-based guidelines for using clopidogrel based on the patient's genotype.[217,218]

For example, the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines recommend patients with a copy of the reduced or loss of function in *CYP2C19* e.g. *CYP2C19**2 or*3 allele are not candidate for taking clopidogrel, and alternative therapies should be commenced.[219] Although the reduced or loss of function in the *CYP2C19* is common, a gain of function allele (*17) has been also reported.[220] However, there is no recommendation based on the gain of function in *CYP2C19* to date. In the Hmong, the frequency of *CYP2C19*2* was found to be higher than Europeans (37.0 vs. 14.5%, $p<0.001$), but the frequency for *CYP2C19*17* was found to be lower than

Europeans (0.2 vs. 22.4%, p<0.001). The frequency of *CYP2C19*3*, however, was indifferent between the Hmong and Europeans (0.2 vs. 0%, p=1.0) (Table 4.1). From the sample population studied, these results translate into 58.4% of Hmong are either homozygote or heterozygote for *CYP2C19*2*. This high percentage of individuals who would not be candidates for clopidogrel represents important information, once validated, to be clinically available for clinicians when deciding on antiplatelet therapy for patients self-reported as Hmong.

Warfarin is a vitamin K epoxide reductase (VKORC1) antagonist and is widely used as an anticoagulant for the treatment of deep venous thrombosis and stroke prevention in patients with atrial fibrillation.[221] Response to warfarin is highly variable with a substantial intra-and inter-patients' variability requiring frequent testing and monitoring to achieve the target INR. Warfarin is a racemic mixture with the S-enantiomer being the active drug that is metabolized by mostly CYP2C9.[222] Changes in the activity of CYP2C9 can greatly influence the response to warfarin and warrant dose adjustment.[223] Moreover, changes in the sensitivity of VKORC1 (drug target) can also influence the dose required to achieve the target INR. In addition to CYP2C9 and VKORC1, residual variability in response to warfarin remained unexplained in select patient populations.[224] Specifically, the response to warfarin in African-American populations have been shown to be significantly affected by CYP4F2 in addition to CYP2C9 and VKORC1.[225] This is to illustrate the fact that there are population-specific genetic variations and the value of multiethnic GWAS to advance precision medicine.

Genetic polymorphisms (SNPs) in *CYP2C9*, *VKORC1* and *CYP4F2* have been

associated with the cumulative/weekly doses of warfarin to achieve the target INR. For example, rs1799853 C>**T (**Cys144Arg) or *CYP2C9*2* is associated with reduced V_{max} of the metabolic activity by 50% relative to the *CYP2C9*1* (normal function) resulting in a reduced clearance of the active drug, S-warfarin, with an average of 20% reduction in warfarin metabolism.[226,227] Consequently, carriers of the *CYP2C9*2* will require lower doses of warfarin compared to *CYP2C9*1* carriers.[227] Another missense SNP rs1057910 A>**C** (Ile359Leu) within the *CYP2C9* noted as *CYP2C9*3*, which is associated with reduced metabolic clearance of S-warfarin relative to *CYP29*2* with an average reduction of 40% relative to the *CYP2C9*1*.[226] Furthermore, the rs9923231 C>**T** within the *VKORC1* promotor region has been associated with decreased expression with the T allele versus the C allele.[226] Therefore, carriers of the T allele will require a lower dose of warfarin compared to the C allele. Finally, the CYP4F2 is a primary liver vitamin K1 oxidase that catalyzes the metabolism of vitamin K1 to hydroxyvitamin K1 and removes vitamin K from the vitamin K cycle. It acts as an important counterpart to VKORC1 in limiting excessive accumulation of vitamin K.[228] The rs2108622 C>**T** (Val433Met) within *CYP4F2* has been also linked with a higher weekly warfarin dosing with the T allele vs. C allele. For instance, a study of Italian patients concluded that the rs2108622 (TT) patients require 5.49 mg/day of warfarin versus 2.93 mg/day for (CC) patients. Analysis of variance of the study indicated that about 7% of mean weekly warfarin dose variance is explained by *CYP4F2* genotype.[229]

In our 235 Hmong participants, the warfarin response complex alleles identified to be important for other populations were significantly different in terms of prevalence,

relative to Europeans (Table 4.1). Specifically, the Hmong have a nearly 3-fold higher prevalence of *CYP2C9*3* compared to Europeans. (21.2% vs. 7.3%, p<0.001) However, *CYP2C9*2* was not detected in our Hmong cohort. This results in 61.7% as *CYP2C9*1/*1*, 37.2% as *CYP2C9*1/*3* and 4.1% *CYP2C9*3/*3*. Moreover, the risk allele (T) of rs9923231 within the *VKORC1* was significantly higher in Hmong than Europeans (88.4% vs. 38.8%, p<0.001). In contrast, the prevalence of the risk allele (T) of rs2108622 within *CYP4F2* was significantly lower in Hmong compared to Europeans (8.9% vs. 29%, p<0.005); however, it was similar to that found in an African cohort (8.9% vs. 8.2%, p=1.00).[45] Given the significant and differential prevalence of the risk alleles in the warfarin response complex in *CYP2C9, VKORC1* and *CYP4F2* between the Hmong and European counterparts, we would predict that on average, Hmong receiving warfarin would likely require a lower maintenance dose of warfarin compared to European-descent patients with the same clinical characteristics.

Assessment of Select Disease Risk Genes in the Hmong

The Hmong have a distinct prevalence of several comorbidities that led the Hmong Advisory Board to bring them to the attention of the research team as potential pharmacogenomics/genetics research. In addition to gout, the Hmong have a 2-fold higher incidence of type 2 diabetes mellitus (T2DM) and higher incidence of head/neck, nasopharyngeal cancers as well as hepatocellular carcinoma.[2,5-7,230] From an exploratory perspective, we estimated the frequencies of two genetic variations within 2 genes, cyclin-dependent kinase inhibitor 2A/B (*CDKN2A/B)* that has strongly linked with the risk of diabetes and nicotinamide adenine dinucleotide phosphate oxidoreductase (*NQO1*)which

has been strongly linked with the risk of cancer.

The *CDKN2A/B* gene is located on chromosome 9(9p21), and provides instructions for making several proteins, acts as a tumor suppressor gene and has been linked to cancer and type 2 diabetes (T2DM).[231-234] The genetic variation rs10811661 C>T adjacent to the *CDKN2A/B* has been linked to impaired fasting blood glucose and the risk of T2DM.[231-233] A meta-analyses of 17 studies with 29,990 cases and 40,977 controls using four genetic models consistently showed an increased risk for T2DM with the T allele relative to the C allele. The allele contrast model had OR = 1.21, 95% CI (1.18-1.24), an additive genetic model had OR = 1.51, 95% CI (1.40-1.63), dominant genetic model had OR = 1.37, 95% CI (1.28-1.47), and the recessive genetic model had OR = 1.25, 95% CI 1.21-1.29).[233] In our Hmong cohort, the prevalence of the risk allele (T) was significantly lower than Europeans (56.1% vs. 83.2%, $p<0.001$) (Table 4.1), which is not in parallel with the high prevalence of T2DM in the Hmong compared to non-Hmong. However, because of the low predictive value of this genetic variant for risk for T2DM, it precludes us to make definitive interpretations from this information.

The *NQO1* gene is located on chromosome 16 (16q22), and encodes NQO1 protein for inactivating and detoxifying carcinogenic compounds[6] and ameliorating some of the drug-induced nephrotoxic side effects of select drugs such as cisplatin.[235] The rs1800566 G>A (Pro187Ser) within *NQO1* causes the NQO1 protein to be rapidly degraded via the ubiquitin dependent pathway shortening the half-life of the enzyme to 1.2 hours, compared to 18 hours for a normal NQO1 protein.[236] The prevalence of the risk allele (A) within the *NQO1* is nearly 3-fold higher in the Hmong than in European, which parallels

the higher incidence of select cancer types in the Hmong population and Han-Chinese in general (Table 4.1).[6,237]

Although we limited our genetic assessment to 2 key SNPs associated with two complex disease states, T2DM and cancer, we acknowledge that the development of diseases like cancer or diabetes are complex and multigenic in nature. However, the different and distinct allele frequencies within genes known to influence the development of the disease cannot be ignored in the overall assessment of the individual's risk stratification for these complex health conditions.

Assessment of Pharmacogenetics of Purine Analogs in the Hmong

Xanthine oxidoreductase (XOR), aldehyde oxidase1 (AOX1) and molybdenum cofactor sulfurase (MOCOS) are the major enzymes involved in purine metabolism, which is the precursor of uric acid (UA) production.[238] Thus, change in the levels of activities in those enzymes may affect baseline UA and possibly the disposition of drugs that are metabolized by those enzymes as well as certain disease states that are modulated by UA production.[166,238]

The XOR is a molybdenum iron-sulfur flavin hydroxylase synthesized as xanthine dehydrogenase (XDH) and predominantly expressed in the liver and gastrointestinal tract. Higher than normal levels of expression of XOR have been also associated with hypoxic conditions, endothelial dysfunction, ischemic injuries and pro-inflammatory cytokines.[239-241] There are two major isoforms of XOR: xanthine oxidase (XO) and xanthine dehydrogenase (XDH). The primary role of XDH is to convert hypoxanthine to xanthine and ultimately to UA. Therefore, a decrease in activity or a loss of function primarily in

XDH can result in a condition known as xanthinuria, which is an excess urinary excretion of the purine base xanthine.[242] Nonetheless, this condition is considered uncommon. Notably, hypoxanthine does not accumulate to an appreciable degree because it is recycled through a salvage pathway by the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT). Two inherited forms of xanthinuria principally result from a deficiency of the enzyme XDH. Specifically, type I xanthinuria, which is the result of an isolated deficiency of XDH[242] and type II xanthinuria, which is characterized by a deficiency of XDH and other related enzymes e.g. AXO1 or MOCOS.[243]

Plasma accumulation and excess urinary excretion of the highly insoluble xanthine due to the deficiency of XDH may lead to arthropathy, myopathy, crystal nephropathy, urolithiasis, or renal failure.[242,243] Inhibiting the activity of XOR is the most common approach to reduce UA production. The most widely used XOR inhibitors are allopurinol and febuxostat. While febuxostat inhibits both isoforms of the enzyme (XO and XDH), allopurinol preferentially inhibit the reduced form of the enzyme (XDH).[156,244] Therefore, knowledge of genetic polymorphisms associated with metabolism of febuxostat and allopurinol can help predict optimal dosing to achieve target SUA. Specifically, allopurinol's activity is mediated by the formation of the long-acting active metabolite, oxipurinol. Thus, being a substrate and an inhibitor of XDH, allopurinol could be considered as a prime example for the effects of genetic polymorphisms within purine genes on the response to drugs.

AOX1 is also a molybdenum iron-sulfur flavin hydroxylase. The exact physiological role of AOX1 remains unclear; however, it is presumed to contribute to the overall purine

metabolism. In addition, AOX1 has been also involved in the metabolism of purine-like drugs such as allopurinol and 6-mercapto purine (6-MP). Molybdenum hydroxylases such as AOX1 and XOR require a final step of maturation to gain enzymatic activity, namely the addition of a terminal sulphido ligand to the molybdenum center.[245] This step is mainly catalyzed by MOCOS, a two-domain protein acting as a homodimer. [245]

Although the effect of genetic polymorphisms in *XOR, AOX1* and *MOCOS* on baseline UA are limited, drugs that are known to be metabolized by the same enzymes can be greatly influenced by these genetic polymorphisms. For example, a study assessed the association between xanthine oxidase inhibitors (XOI) and genetic polymorphisms within 6 genes associated with the metabolism and clearance of XOIs in 100 patients with gout and stable XOI dose as the primary outcome. [166] While being a retrospective in design and lacking corrections for making multiple comparisons, the study identified several SNPs to have significant associations with different tiers of allopurinol or allopurinol-equivalent stable doses (< 300mg/d, 300mg/d and > 300mg/d). Specifically, the intronic rs75995567 T>C within *AOX1* was found to be significantly (p=0.031) associated with using higher stable doses of allopurinol (> 300mg/d).[166] Generally, allopurinol is uncommonly prescribed in doses greater than 300mg/day.

Additionally, Kurzawski et al[246] enrolled 156 renal transplant recipients with at least 12 months of graft survival and receiving triple immunosuppressive therapy (azathioprine (AZA), cyclosporine and prednisone) to investigate the effects of SNPs in *XDH*, *AOX1* and *MOCOS* genes in relation to clinical parameters and drug adverse events.[246] The study identified that individuals carrying the missense rs55754655 A>G (Asn1135Ser) within

AOX1 required a significantly higher dose of AZA compared with non-carriers suggesting decrease formation of the active metabolites of 6-MP. However, the missense rs594445 C>A (His703Asn) within *MOCOS* resulted in a lower activity requiring a significantly lower dose of AZA dose compared with non-carriers.[246]

Given the interplay of MOCOS and AOX1 activities in terms of their roles in AZA dosing needs and the shared metabolic pathway between AZA and allopurinol, it is plausible that decreased function in MOCOS can reduce XOR activity affecting baseline UA and allopurinol metabolism.[166] Therefore, it could be theorized that the production of oxipurinol and UA will be decreased in participants with the risk alleles in either *MOCOS, AOX1 or XDH*.

To assess whether the risk alleles in key purine metabolizing genes: *XDH, AOX1,* or *MOCOS* may be contributing to the higher baseline SUA or higher incidence of gout in Hmong than non-Hmong, the prevalence of 3 previously identified SNPs[246] (rs4407290, rs3731722, rs594445) were compared to CEU. Notably, two SNPs (rs4407290 G>A within *XDH* and rs594445 C>A within *MOCOS*) out of the 3 SNPs were identified by Kurzawski et al.[246] However, the third SNP, rs3731722 A>G (His1297Arg) within *AOX1*, was of a particular interest as it's been shown to be significantly associated with allopurinol dose in a different study.[166] Using Chi-Square test or fisher's exact test when appropriate, only the frequency of the rs594445 within *MOCOS* was found to be significantly different in our Hmong cohort than CEU (Table 4.2). Within our Hmong cohort of 235 adults, the estimated prevalence of risk allele (A) was higher in CEU than Hmong (27.3% vs. 10.1%, $p<0.001$). Notably, the A allele for rs594445 C>A is associated

with a lower enzymatic activity of MOCOS relative to the C allele. This finding suggests that the Hmong have a higher frequency of the normal activity of MOCOS, which is an important cofactor for the normal activity of AOX1 and XOR relative to CEU. This observation may also in part, explain the higher baseline SUA in the Hmong men and women (7.14 and 5.97mg/dL)[79] relative to the average US men and women (6.14 and 4.87mg/dL),[11] respectively. Whether this missense rs594445 C>A is associated with developing gout is yet to be determined. However, higher baseline SUA is the hallmark for developing gout.

Assessment of Uric Acid Genetics in the Hmong

We previously reported on the prevalence of select 8 SNPs within 5 genes known to influence the elimination and reabsorption of uric acid in the Hmong in relation to other populations.[79] In this addendum, we expand our analysis to include additional SNPs within genes using the same approach highlighted in our previous work and the approach summarized for the selection of SNPs in pathway pharmacogenetics.[247] Specifically, the prevalence of rs2242206 G>T within *SLC16A9*, rs17300741 within *SLC22A11* G>A, rs780094 C>T within *GCKR* and rs742132 G>A within *LRRC16A* were estimated in the Hmong then compared to CEU and CHB (Tables 4.2-3).

SLC16A9 is located on chromosome 10 and encodes the monocarboxylic acid transporter 9 (MCT9). Although the exact role of MCT9 in UA disposition is not well understood, it is believed to act as a UA and carnitine efflux transporter.[80,248] The meta-analysis by Kolz et al[80] identified an association between the intronic rs12356193 G>A within *SLC16A9* with increased SUA. Metabolomics studies have also shown that

rs12356193 within *SLC16A9* is associated with DL-carnitine and propionyl L-carnitine. Moreover, the DL-carnitine and propionyl-L-carnitine are strongly associated with UA.[80]

In our Hmong cohort, we genotyped for the rs2242206 G>T within *SLC16A9* due to being a missense SNP (K285T) and more prevalent in a concordant population group to the Hmong such as CHB. Furthermore, both SNPs (rs2242206 and rs12356193) are in high linkage disequilibrium. Again, the prevalence of the risk allele (T), which is associated with the dysfunctional transporter, was significantly higher in the Hmong than CEU (51.5 vs 22.7%, $p<0.001$). Given the effect of this transporter on UA, reduced function of the MCT9 transporter may possibly lead to a substantial accumulation of UA due to decreased excretion. Indirect support of this rationale may come from a study by Nakayama et al[84] which demonstrated that the presence of this T for rs2242206 G>T within *SLC16A9* in Japanese patients with gout has been significantly associated with UA overproduction, a gout sub-type sometimes referred to as renal overload, but not specifically leading to gout susceptibility.

SLC22A11is located on chromosome 11 and encodes the organic anion transporter 4 (OAT4), which is primarily expressed on the apical side of the proximal convoluted tubule. Relative to the URAT1 transporter, the OAT4 transporter plays a modest role in UA reabsorption back into systemic circulation and is inhibited by select uricosuric drugs.[249] The effect of genetic polymorphism rs17300741G>A within OAT4 has been associated with a significantly increased baseline SUA level within CEU.[80] It has also demonstrated a borderline significant level of association with subjects of the renal underexcretion gout sub-type but not gout susceptibility in Japanese.[85] In the Hmong, the

prevalence of the risk (A) was found to be nearly 2-fold higher compared to CEU (93.7 vs 46.5%, p<0.001). This observation further suggests that the prevalence of risk alleles in the Minnesota Hmong appear to parallel the observed higher prevalence of gout and increased risk of hyperuricemia. Given the putative physiological role of this transporter, it is plausible to that individuals with the (A) allele for rs17300741G>A could have the normal to increased function of the transporter relative to the individuals with the (G) allele. If confirmed, it may explain an increased ability for UA reabsorption thereby enhancing the risk for developing hyperuricemia and gout.

Additional genes in this addendum have been previously identified by Kolz et al[80], validated by others and assessed in our present Hmong cohort. These genes are not transporter genes rather structural and enzymatic genes indirectly related to baseline SUA. For instance, the *LRRC16A* is a protein coding gene for CARMIL1 (Capping Protein Regulator and Myosin 1 Linker 1) located on chromosome 6. Diseases associated with decreased function of CARMIL1 include elevated SUA and gout.[80,209] Furthermore, variation in the GCKR (glucokinase regulatory protein) activity has been shown to be associated with increased SUA and gout,[205,209] while being also associated with lipid and glucose metabolism.

The intronic SNP rs742132 G>A within *LRRC16A* has been linked to increased SUA in CEU[80] and increased risk for gout in Japanese.[209] The frequency of the risk allele (A) in the Hmong was 86.3% compared to CEU 69.7% (p<0.001). Again, the Hmong appear to have a genetic basis for being at higher risk for both increased SUA and gout compared to CEU. The intronic SNP rs780094 C>T within *GCKR* has been also linked to

increased SUA in CEU[80] and increased risk for gout in Han Chinese males.[205] The frequency of the risk allele (T) for rs780094 in the Hmong was 28.6% compared with 40.9% (p=0.003) seen in CEU. Although the frequency of this risk allele observed the Hmong was not directionally consistent with their increased risk of hyperuricemia or gout, we believe this gene is directly associated with glucose and lipid metabolism.

In comparison to CHB, the Hmong were found to have a higher prevalence of the risk allele (C) of rs594445 C>A within *MOCOS* (89.9% vs. 72.3%, p<0.001) and the risk allele (A) of rs742132 G>A within *LRRC16A* (86.3% vs. 75.7%, p=0.001). In contrast, the Hmong were found to have a lower prevalence of the risk allele (T) of rs780094 C>T within *GCKR* compared to CHB (28.6% vs. 59.2%, p<0.001) (Table 4).

In summary, apart from rs780094C>T within the *GCKR*, the prevalence of the risk alleles listed in Table 4.2 are consistent with the increased risk of hyperuricemia and gout in the Hmong compared to CEU. Despite the Hmong typically being classified as a subset of CHB, they manifest a higher prevalence of the risk alleles associated with hyperuricemia and gout compared to reference cohorts of CHB. Collectively, our findings listed in Tables 4.2-3 are consistent with our earlier report on the prevalence of 8 risk alleles in the Hmong compared with CEU and CHB.

Assessment of hyperuricemia or gout risk alleles by Sex in Hmong

The global prevalence of hyperuricemia and gout is more common in men than women. Specifically, Hmong men have a much higher prevalence of gout compared to CEU (11.5 vs 4.1%).[17] The prevalence of gout among Hmong women, however, is similar to that reported in CEU (1.9 vs. 1.9%).[17] Consequently, assessing the frequencies of the

risk alleles between Hmong men and women is warranted may lead to a genetic signature for this observation. From our assessment of allele frequencies in our Hmong cohort,[79] we identified that the rs1183201A>T within the *SLC17A1* was significantly different between Hmong women and men (Table 4.4-5). *SLC17A1* encodes the sodium-dependent phosphate cotransporter type 1 (NPT1), which is a UA efflux transporter.[204] Stratified by sex, we found the frequency of risk allele (T) in Hmong males was modestly higher than in Hmong women (88.5 vs 80.4, p=0.026) (Tables 4.2-3). Since rs1183201 A>T within the *SLC17A1* is associated with the risk of gout,[205] SUA levels,[80] this finding suggests that genetic polymorphisms in the efflux NPT1transporter may play a role in Hmong males' susceptibility for gout compared to women. Indeed, the prevalence of the risk (T) for the rs1183201 A>T is 50% in CEU versus 83.6% (<0.001) in the Hmong.

A meta-analysis of sex specific results using a CEU population of 12,328 males and 15,813 females did not show any additional genome-wide significant locus to have sex-specific effects on SUA levels.[80] However, it identified that the gender specific effect for the minor allele (G) of rs734553 T>G within the SLC2A9, resulting in a 2-fold larger effect size on SUA concentrations in women compared to men. For *ABCG2*, however, the effect of the minor allele (T) of rs2231142 G>T demonstrated a larger effect on UA concentrations in men compared to women. For the other loci, effect sizes did not significantly differ by gender.[80]

In summary, along with the non-genetic factors (diet and lifestyles) and sex-hormonal differences between males and females, genetic variations such as SNPs in uric acid transporter encoding genes can differentially influence the risk of developing gout

between sexes.

Genetic Assessment of Hyperuricemia in the Hmong

In our earlier work,[79] our SNP by SNP analysis did not show any association between the 8 SNPs in the 5 targeted genes and the risk of hyperuricemia. However, a limitation of our previously conducted analysis was the use of a very conservative definition of hyperuricemia (SUA $\geq$ 6mg/dL). In this analysis, we use serum uric acid saturation threshold for defining hyperuricemia (SUA$\geq$ 6.8mg/dL) and Fisher exact test with $p<0.05$ for significance. This analysis resulted in one SNP rs1183201 within *SLC17A1* to be significantly associated with clinical definition of hyperuricemia as shown in Table 4.6. Though this is consistent with previously published data on the association of rs1183201 A>T within the *SLC17A1 and* increased SUA, we note that rs1183201 A>T was not in HWE in the participants with SUA $\geq$ 6.8mg/dL. In the allele contrast model analysis, however, the rs1183201 A>T was not significantly associated with hyperuricemia. As previously described, the rs1183201 A>T is a missense SNP with the risk allele T being associated with a dysfunctional UA efflux transporter NPT1 and has been associated with elevated baseline SUA and increased risk for gout while being more prevalent in Hmong males than Hmong women.

Assessment of Baseline SUA Predictors in the Hmong

Using multiple linear regression with serum uric acid as the dependent variable and 14 SNPs within 12 genes, eCrCl measured by Cockcroft-Gault and sex as the independent variables, the model explained 52% (P=0.02) of SUA variability. While eCrCl was a significant predictor for SUA ($p<0.001$), the rs3733591 within *SLC2A9* achieved

statistical significance (*P*=0.046) and rs505802 within *SLC22A12* was borderline significant (*P*=0.058) (Table 4.7).

Using a hierarchical multiple linear regression approach to identify predictors of baseline SUA variability, we analyzed 50 out of the 57 participants with known baseline SUA, sex, eCrCl, and complete genotypes information for 14 SNPs within 12 genes. With baseline SUA as the dependent variable and controlling for eCrCl, the independent variables i.e. sex and genotypes were tested in a stepwise regression fashion using probability criteria of F-to-enter $\leq$0.05 and probability criteria-of F-to-remove $\geq$0.10.

In addition to eCrCl, gender was the only significant predictor of baseline SUA. In combination, gender and eCrCl explained 34% (*P*=0.002) of the variability around baseline SUA. Although physiologically consistent with the impact of sex and kidney function on the risk of developing hyperuricemia or gout the effect size of these genetic factors on baseline SUA are relatively small. Consequently, our limited sample size precludes us from identifying any association of SNPs with baseline SUA in our Hmong cohort after adjusting for confounding variables such as kidney function and sex.

Association of Uric Acid Levels with Cardiovascular Diseases in the Hmong

Out of the 57 participants with measured SUA, available anthropometrics data (waist circumference, systolic and diastolic blood pressure), levels of HDL, and known status of taking antidiabetic medications, we identified that 26.3% (15/57) of the study participants have metabolic syndrome (MS) based on NCEP/ATPIII guidelines.[250] The mean (±SD) of SUA for participants with MS was 7.1 (±1.8) mg/dL . Participants (10/57) without risk factors for MS, however, did not have statistically different mean SUA of 6.1

(±2.2) than those with MS. Although our results remain consistent with the estimated prevalence of MS to be 18% in subjects with SUA < 6mg/dL,[20] it remains lower than the prevalence of MS in individuals with mean SUA of 7.1 mg/dL.[19] Evidently, the percentage of participants with metabolic syndrome could have increased should we have a full metabolic and lipid panel on those participants. Notably, the same 57 study participants had measured non-fasting glucose levels thus we did not use the measured glucose levels for assessing the risk for MS.

Consistent with the increased risk for developing MS, SUA levels have been also associated with the different components that make up the MS. In addition, levels of SUA were negatively associated with eCrCl and it is believed that higher SUA level is a major risk factor for increased incidence of chronic kidney diseases.[11] Analysis of our data on 57 Hmong with known SUA, depict a trend for associations between SUA with other cardiovascular risk factors consistent with the literature and other epidemiological studies. Specifically, SUA levels were positively associated with SBP (P=0.055), and total cholesterol (p=0.055) (Figure 4.1-2); however, they were negatively associated with HDL (P=0.071) and eCrCl (P<0.001) (Figure 4.3-4).

In summary, we document the trend of association between SUA levels and the risk of MS as well as cardiovascular disease risk factors. Although our sample size was small to show a significant association, we note the consistent trends of increased SUA levels with the risk factors known to increase the incidence of cardiovascular diseases.

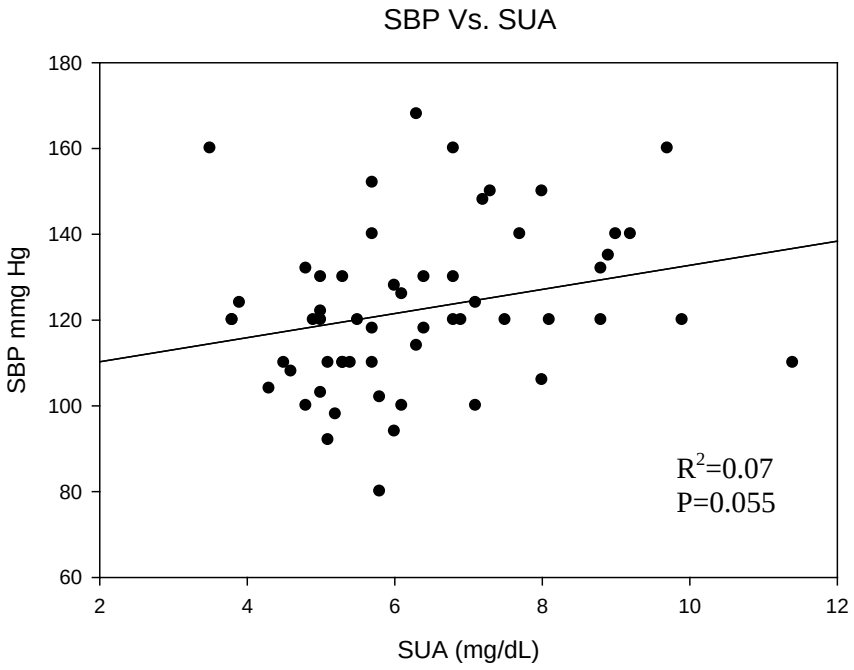
Conclusions

The Minnesota Hmong have a distinct allele frequencies of clinically validated

pharmacogenes associated with key cardiovascular drugs compared to European descents. In addition, we note a distinct prevalence of two key risk allele genes associated with T2DM and decreased detoxification of carcinogen compounds in the Hmong relative to European descents. Consistent with the high prevalence of hyperuricemia and gout in the Hmong, select allele frequencies of genes involved in purine metabolism and uric acid disposition are higher in the Hmong compared with CEU and CHB and directionally consistent with increased risk of hyperuricemia and gout. With hyperuricemia and gout being more prevalent in Hmong than non-Hmong, we also identified that Hmong men tend to have a significantly higher prevalence of the risk allele (T) of rs1183201 A>T within *SLC17A1* than Hmong women while remaining substantially higher than CEU overall.

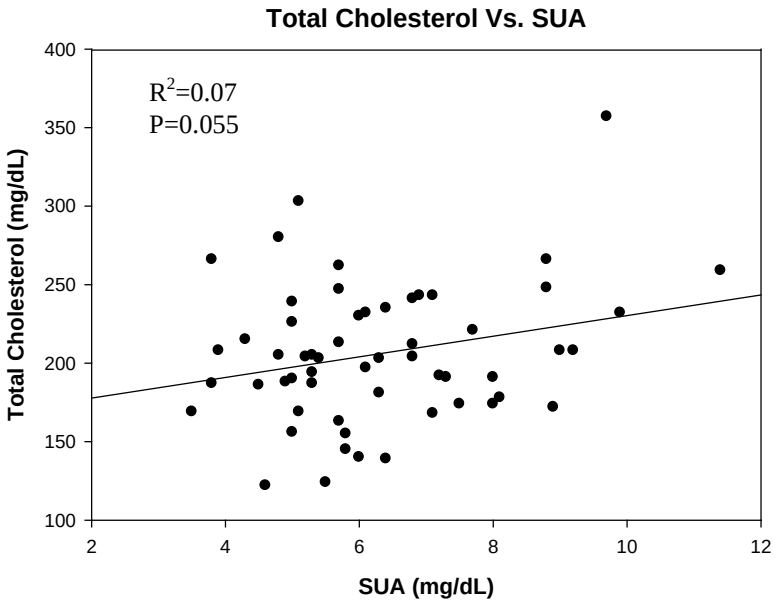
Finally, SUA levels have been long known to be strongly associated with hypertension, chronic kidney disease, diabetes, dyslipidemia and metabolic syndrome in large epidemiological studies. In our sample of 57 participants with known SUA levels, we observed similar patterns of association in our Hmong cohort. For instance, participants with metabolic syndrome had a higher level of SUA than those without; however, the difference was not significant. Additionally, we observed borderline significant associations between SUA levels and systolic blood pressure, eCrCl, HDL and total cholesterol.

Figure 4:1 Association between SUA levels and SBP



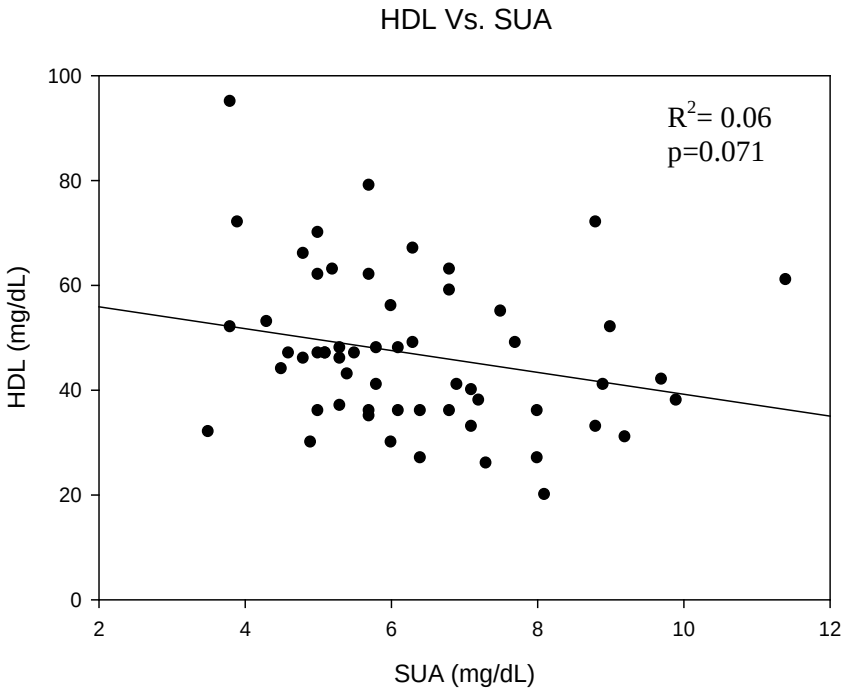
SBP= Systolic Blood Pressure
SUA= Serum Uric Acid

Figure 4:2 Association between SUA and total cholesterol



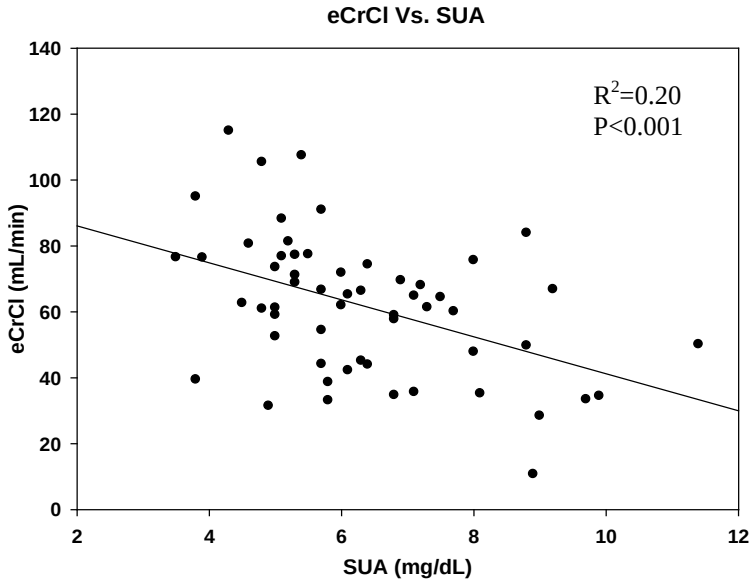
SUA: Serum Uric Acid

Figure 4:3 Association between SUA and HDL



HDL: High Density Lipoprotein
SUA: Serum Uric Acid

Figure 4:4 Association between SUA and eCrCl



eCrCl: estimated Creatinine Clearance
SUA: Serum Uric Acid

Table 4:1 Frequencies of Select VIP and Disease Risk Genes in Hmong and Europeans							
		Hmong (N=235)			**Europeans (1000 Genome Project)** [45]		
Gene (SNP)	Allele	Allele N (%)	Genotype	N (%)	Allele N (%)	Genotype	N (%)
SLCO1B1 (rs4149056) T>C‡†	T **C**	446 (97.4) 12 (2.6)	TT CT CC	217 (94.8) 12 (5.2) 0 (0)	844 (83.9) 162 (16.1)	TT CT CC	351 (69.8) 142 (28.2) 10 (2.0)
*CYP2C19*2* (rs4244285) G>A‡†	G **A**	276 (63.0) 162 (37.0)	GG AG AA	90 (41.6) 91 (42.9) 34 (15.5)	860 (85.5) 146 (14.5)	GG GA AA	363 (72.2) 134 (26.6) 6 (1.2)
*CYP2C19*3* (rs4986893) G>A	G **A**	457 (99.8) 1 (0.2)	GG AG AA	228 (99.6) 1 (0.4) 0 (0)	1006 (100) 0	GG AG AA	503 (100) 0 0
*CYP2C19*17* (rs12248560) C>T‡†	C **T**	461 (99.8) 1 (0.2)	CC CT TT	230 (99.6) 1 (0.4) 0 (0)	781 (77.6) 225 (22.4)	CC CT TT	300 (59.6) 181 (36.0) 22 (4.4)
VKORC1 (rs9923231) C>T‡†	C **T**	54 (11.6) 410 (88.4)	CC CT TT	5 (2.2) 44 (19.0) 183 (78.9)	616 (61.2) 390 (38.8)	CC CT TT	192 (38.2) 232 (46.1) 79 (15.7)
*CYP2C9*2* (rs1799853) C>T‡†	C **T**	458 (100) 0 (0)	CC TT	229 (100) 0 (0)	881 (87.6) 125 (12.4)	CC CT TT	389 (77.3) 103 (20.5) 11 (2.2)
CYP2C9*3 (rs1057910) A>C‡†	A **C**	350 (78.8) 94 (21.2)	AA CA CC	137 (61.7) 76 (34.2) 9 (4.1)	933 (92.7) 73 (7.3)	AA CA CC	431 (85.7) 71 (14.7) 1 (0.2)
CYP4F2 (rs2108622) C>T‡†	C **T**	419 (91.1) 41 (8.9)	CC CT TT	194 (84.3) 31 (13.5) 5 (2.2)	714 (71.0) 292 (29.0)	CC CT TT	261 (51.9) 192 (35.2) 50 (9.9)
NQO1 (rs1800566) G>A‡†	G **A**	176 (38.9) 276 (61.1)	GG GA AA	29 (12.8) 118 (52.2) 79 (35)	794 (78.9) 212 (21.2)	GG GA AA	315 (62.6) 164 (32.6) 24 (4.8)
CDKN2A/B (rs10811661) C>T‡†	C **T**	202 (43.9) 258 (56.1)	CC CT TT	46 (20.0) 110 (47.8) 74 (32.2)	169 (16.8) 837 (83.2)	CC CT TT	16 (3.2) 137 (27.2) 350 (69.6)

‡† indicates (allele and genotype) statically significant difference between Hmong and European (P<0.005 for significance)

Note: many of those SNPs deviated from HWE due to monomorphism or low frequency

Bolded allele indicates the risk allele

VIP: Very Important Pharmacogenes

Table 4:2 Frequencies of Purine and Uric Acid Modulator Genes in CEU and Hmong						
Gene (SNP)	**Genotype**	**CEU N (%)** [45]		**Hmong N (%)**		***P*-value**
XDH *(rs4407290) G>**A*** *Chromosome 2*	GA GG Total	5 94	5.1 94.9	9 218 227**	4.0 96.0	0.75
*AOX1** *(rs3731722) A>**G*** *Chromosome 2*	AG AA Total	9 90	9.1 90.9	21 208 229**	9.2 90.8	1.00
*MOCOS** *(rs594445) C>**A*** *Chromosome 18*	AA CA CC Total	7 40 52	7.1 40.4 52.5	5 36 187 228	2.2 15.8 82.0	**<0.001**
*SLC16A9** *(rs2242206) G>**T*** *Chromosome 10*	GG GT TT Total	61 31 7	61.6 31.3 7.1	55 114 62 231	23.8 49.4 26.8	**<0.001**
*SLC22A11 (rs17300741) G>**A*** *Chromosome 11*	AA AG GG Total	23 46 30	23.2 46.5 30.3	202 27 1 230	87.8 11.7 0.4	**<0.001**
LRRC16A *(rs742132) G>**A*** *Chromosome 6*	AA GA GG Total	46 46 7	46.5 46.5 7.1	171 55 4 230	74.3 23.9 1.7	**<0.001**
GCKR *(rs780094) C>**T*** *Chromosome 2*	CC TC TT Total	34 49 16	34.3 49.5 16.2	117 96 18 231	50.6 41.6 7.8	0.007

Bolded allele indicates the risk allele
* Missense SNP
** Not in a Hardy-Weinberg Equilibrium
P<0.006 for significance
Note: many of those SNPs deviated from HWE due to monomorphism or low frequency

Table 4:3 Frequencies of Purine and Uric Acid Modulator Genes in CHB and Hmong						
Gene (SNP)	**Genotype**	**CHB N (%)[45]**		**Hmong N (%)**		*P*-value
XDH *(rs4407290) G>**A*** *Chromosome 2*	GA GG Total	10 93	9.7 90.3	9 218 227**	4.0 96.0	0.044
*AOX1** *(rs3731722) A>**G*** *Chromosome 2*	GG AG AA Total	2 12 89	1.9 11.7 86.4	0 21 208 229**	0 9.2 90.8	0.083
*MOCOS** *(rs594445) C>**A*** *Chromosome 18*	AA CA CC Total	8 41 54	7.8 39.8 52.4	5 36 187 228	2.2 15.8 82.0	**<0.001**
*SLC16A9** *(rs2242206) G>**T*** *Chromosome 10*	GG GT TT Total	16 55 32	15.5 53.4 31.1	55 114 62 231	23.8 49.4 26.8	0.227
SLC22A11 *(rs17300741) G>**A*** *Chromosome 11*	AA AG GG Total	89 13 1	86.4 12.6 1.0	202 27 1 230	87.8 11.7 0.4	0.742
LRRC16A *(rs742132) G>**A*** *Chromosome 6*	AA GA GG Total	57 42 4	55.3 40.8 3.9	171 55 4 230	74.3 23.9 1.7	**0.002**
GCKR *(rs780094) C>**T*** *Chromosome 2*	CC TC TT Total	16 52 35	15.5 50.5 34.0	117 96 18 231	50.6 41.6 7.8	**<0.001**

Bolded allele indicates the risk allele

* Missense SNP

** Not in a Hardy-Weinberg Equilibrium (N=235)

P<0.006 for significance

Table 4:4 Allele Frequencies Stratified by Sex in Hmong						
Gene (SNP)	**Allele***	**Male (N=105)**		**Female (N=130)**		P-value
		N	**%**	**N**	**%**	
SLC17A1	**T**	184	88.5	201	80.4	**0.026**
(rs1183201)	A	24	11.5	49	19.6	
PDZK1	A	72	34.6	79	31.6	0.492
(rs12129861)	**G**	136	65.4	171	68.4	
SLC22A11	**A**	198	95.2	233	92.5	0.230
(rs17300741)	G	10	4.8	19	7.5	
ABCG2	A	109	52.4	138	55.2	0.548
(rs2231137)	**G**	99	47.6	112	44.8	
ABCG2	**A**	75	35.7	90	35.7	1.00
(rs2231142)	C	135	64.3	162	64.3	
SLC16A9	**T**	103	49.5	135	53.1	0.438
(rs2242206)	G	105	50.5	119	46.9	
ABCG2	G	186	89.4	223	87.8	0.584
(rs2725220)	**C**	22	10.6	31	12.2	
AOX1	**A**	199	95.7	238	95.2	0.806
(rs3731722)	G	9	4.3	12	4.8	
SLC2A9	**T**	208	99.5	243	98.0	0.226
(rs11942223)	C	1	0.5	5	2.0	
SLC2A9	A	118	56.7	148	60.2	0.458
(rs3733591)	**G**	90	43.3	98	39.8	
SLC2A9	**T**	142	69.3	173	68.7	0.708
(rs1014290)	C	60	29.7	79	31.3	
XDH	A	4	1.9	5	2.0	1.00
(rs4407290)	**G**	204	98.1	241	98.0	
SLC22A12	T	66	32.4	95	38.0	0.210
(rs505802)	**C**	138	67.6	155	62.0	
MOCOS	A	15	7.3	31	12.4	0.071
(rs594445)	**C**	191	92.7	219	87.6	
LRRC16A	**A**	181	87.0	216	85.7	0.689
(rs742132)	G	27	13.0	36	14.3	
GCKR	**T**	59	28.1	73	29.0	0.841
(rs780094)	C	151	71.9	179	71.0	
HLA-B	G	206	98.1	246	98.4	1.00
(rs9263726)	**A**	4	1.9	4	1.6	

*Bolded allele indicates the risk allele

Risk allele is defined as the allele that is not associated with reduced SUA based on the GWAS by Kolz et al [80]

Table 4:5 Genotype Frequencies by Sex in Hmong						
Gene (SNP)	**Genotype**	**Male (N=105)**		**Female (N=130)**		*P*-value
		N	**%**	**N**	**%**	
SLC17A1 (rs1183201)	TT	83	79.8	79	63.2	**0.0104**
	AT	18	17.3	43	34.4	
	AA	3	2.9	3	2.4	
PDZK1 (rs12129861)	AA	12	11.5	15	12.0	0.554
	AG	48	46.2	49	39.2	
	GG	44	42.3	61	48.8	
SLC22A11 (rs17300741)	AA	95	91.3	107	84.9	0.081
	AG	8	7.7	19	15.1	
	GG	1	1.0	0	0.0	
ABCG2 (rs2231137)	AA	28	26.9	36	28.8	0.778
	AG	53	51.0	66	52.8	
	GG	23	22.1	23	18.4	
ABCG2 (rs2231142)	AA	14	13.3	18	14.3	0.951
	CA	47	44.8	54	42.9	
	CC	44	41.9	54	42.9	
SLC16A9 (rs2242206)	TT	24	23.1	38	29.9	0.477
	GT	55	52.9	59	46.5	
	GG	25	24.0	30	23.6	
ABCG2 (rs2725220)	GG	82	78.8	99	78.0	0.394
	CG	22	21.2	25	19.7	
	CC	0	0.0	3	2.4	
AOX1 (rs3731722)	TT	95	91.3	113	90.4	0.823
	TC	9	8.7	12	9.6	
	CC	0	0.0	0	0.0	
SLC2A9 (rs3733591)	AA	34	32.7	43	35.0	0.651
	GA	50	48.1	62	50.4	
	GG	20	19.2	18	14.6	
SLC2A9 (rs1014290)	TT	7	6.9	9	7.1	0.934
	CT	46	45.5	61	48.4	
	CC	48	47.5	56	44.4	
XDH (rs4407290)	AA	0	0.0	0	0.0	1.00
	GA	4	3.8	5	4.1	
	GG	100	96.2	118	95.9	
SLC22A12 (rs505802)	TT	11	10.8	23	18.4	0.278
	CT	44	43.1	49	39.2	
	CC	47	46.1	53	42.4	
MOCOS (rs594445)	AA	1	1.0	4	3.2	0.262
	CA	13	12.6	23	18.4	
	CC	89	86.4	98	78.4	

P<0.05 indicates statistically significant

Table 4:6 Risk of Hyperuricemia by Genotype				
SLC2A9 (rs734553) *	TT	GT	GG	P-value
SUA <6.8 mg/dL (n)	34	0	0	NS
SUA ≥6.8 mg/dL (n)	19	1	0	
SLC2A9(rs1014290)	TT	TC	CC	
SUA <6.8 mg/dL (n)	14	17	1	NS
SUA ≥ 6.8mg/dL (n)	7	10	2	
SLC2A9 (rs11942223) *	TT	TC	CC	
SUA <6.8 mg/dL (n)	33	0	0	NS
SUA ≥ 6.8mg/dL (n)	17	1	0	
PDZK1(rs12129861) *	AA	AG	GG	
SUA <6.8 mg/dL (n)	3	12	18	NS
SUA ≥ 6.8 mg/dL (n)	2	8	8	
ABCG2 (rs2231142) *	AA	CA	CC	
SUA <6.8 mg/dL (n)	2	12	19	NS
SUA ≥ 6.8 mg/dL (n)	3	6	11	
SLC22A12 (rs505802) *	TT	TC	CC	
SUA <6.8 mg/dL (n)	6	15	11	NS
SUA ≥ 6.8 mg/dL (n)	2	9	9	
SLC2A9 (rs3733591) *	GG	GA	AA	
SUA 6.8 mg/dL(n)	4	17	11	NS
SUA ≥ 6.8mg/dL (n)	3	10	5	
SLC17A1 (rs1183201)	**TT**	**AT**	**AA**	
SUA <6.8 mg/dL (n)	**20**	**12**	**1**	**0.02**
SUA ≥ 6.8mg/dL (n)*	**17**	**1**	**1**	

Hyperuricemia was defined as SUA ≥ 6.8 mg/dL
* Indicates the SNP was not in a Hardy-Weinberg Equilibrium (N=57)
P<0.05 indicates statistical significance using Chi-Square or Fisher's exact test
NS: Not Significant

Table 4:7 Baseline SUA Multiple Linear Regression Summary Statistics*							
	Coefficients	***SE***	***P-value***	***R^2***	***Adj. R^2***	***SE***	***Sig. F***
Intercept	9.431	2.713	0.001	0.54	0.32	1.3	0.02
eCrCl (mL/min)	**-0.039**	**0.011**	**0.001**				
Gender	-0.772	0.449	0.095				
SLC17A1_ rs1183201	0.187	0.466	0.690				
PDZK1_ rs12129861	-0.404	0.332	0.232				
SLC22A11_ rs17300741	0.657	0.594	0.277				
ABCG2_ rs2231137	0.165	0.487	0.737				
ABCG2_ rs2231142	0.016	0.639	0.980				
SLC16A9_ rs2242206	-0.221	0.349	0.532				
XDH_ rs2364916	0.034	0.313	0.915				
ABCG2_ rs2725220	-0.008	0.726	0.991				
SLC2A9_ rs3733591	**-0.721**	**0.348**	**0.046**				
SLC2A9_ rs1014290	-0.375	0.375	0.324				
SLC22A12_ rs505802	0.607	0.309	0.058				
MOCOS_ rs594445	-0.473	0.557	0.402				
LRRC16A_ rs742132	0.596	0.406	0.152				
GCKR_ rs780094	0.020	0.362	0.956				

*Dependent variable was baseline serum uric acid (N=50)
Using an additive model when coding the SNPs
P<0.05 indicates statistical significance

Chapter 5

The Pharmacodynamics, Pharmacokinetics, and Pharmacogenomics of Allopurinol in Hmong Adults with Gout or Hyperuricemia: The **G**enetics **O**f Hyper**U**ricemia and Gout **T**herapy in **H**mong (GOUT-H) Study

Introduction

Gout, the most common inflammatory arthritic condition, is caused by deposition of monosodium urate crystals in distal joints.[140] Hyperuricemia, the hallmark for developing gout, is strongly associated with cardiovascular diseases[251] including hypertension[56], metabolic syndrome[54] and type 2 diabetes mellitus.[186] The prevalence of gout and hyperuricemia are rising globally and within the US, in part due to changes in lifestyles, increasing longevity and obesity. In addition, race, ethnicity and genetics may contribute to developing hyperuricemia and gout.[80,81,208] For example, the prevalence of risk alleles associated with hyperuricemia and gout vary by ancestry in a manner consistent with the prevalence of gout.[81,205] For the most part, genetic polymorphisms linked to the development of hyperuricemia and gout are found within genes encoding uric acid (UA) transporters including *ABCG2*, *SLC2A9*, *SLC16A9*, *SLC17A1*, *SCL22A11*, and *SLC22A12*. Other genes that may also play a role include *PDZK1*, *LRRC16A* and *GCKR*.[80,81] While the overall prevalence of gout in the US is 3.9%,[11] select ethnic and racial groups may be up 10.4% [10,11] A prime example is the Hmong.[17]

The Hmong are a unique Asian sub-population numbering over 250,000 in the US with over 64,000 living in Minnesota.[252] In addition to a 2-fold increased risk of gout,[17] Minnesota Hmong manifest gout at a younger age with more complications and have up to 5-fold increased risk of gout-associated co-morbidities compared to non-Hmong.[24,34] Furthermore, select hyperuricemia and gout associated risk alleles are more prevalent in the Hmong compared to Caucasian (CEU) and Han-Chinese (CHB) populations.[24,79] Collectively, these findings strongly suggest genetics play a role in the risk for

hyperuricemia and gout in the Hmong, and possibly their response to medications, diet and lifestyle factors.

Allopurinol is the most prescribed urate-lowering therapy (ULT) in the management of gout.[143,181] Rapidly absorbed, allopurinol is metabolized to the active metabolite, oxipurinol. Oxipurinol is renally eliminated, yet undergoes extensive reabsorption via the URAT1 transporter encoded by *SLC22A12*.[253] The high variability in plasma oxipurinol concentrations following allopurinol dose[254] suggests URAT1, specifically, the single nucleotide polymorphism (SNP) rs505802 T>C, to be a probable source of variability in response to allopurinol. In part, this variant may contribute to the observed high failure rates to achieve target serum uric acid (SUA) levels in patients treated with allopurinl.[163,255] Consequently, we sought to specifically target the rs505802 T>C within *SLC22A12* as a potential contributor to the pharmacokinetics and pharmacodynamics of allopurinol.

The higher prevalence of gout and gout-risk alleles in the Hmong[17,79] provided the basis to conduct the GOUT-H study (clinicaltrial.gov, NCT02371421). Our central hypothesis is the rs505802 T>C within *SLC22A12* affects the PK and PD of allopurinol in Hmong adults. The objectives of this study were to assess the effectiveness of allopurinol in lowering SUA in Hmong adults with hyperuricemia or gout and to quantify the impact of key SNPS on the urate lowering response to allopurinol.

Materials and Methods:

Study Design

This genetically-guided, prospective, open-labeled, pilot study utilized the principles of community-based participatory research (CBPR) and capitalized on an

established partnership with the Hmong community.[34] A ten-member Hmong Gout Research Board, consisting of the 3 study investigators plus 7 Hmong professionals and community researchers, conducted the study. The Hmong Board members gave input into the cultural appropriateness of study design, recruitment methods, and consent materials; two members actively recruited and consented participants while assisting in sample collection and completion of questionnaires from both Hmong and English speaking participants. All study materials were approved by the Human Research Protection Program at the University of Minnesota Institutional Review Board (IRB #1408M53223).

Study Participants and Study Visits

We enrolled male and female Hmong adults ≥ 18 years old with SUA ≥ 6mg/dL, or history of gout documented with use of ULT, or SUA <6mg/dL with a history of 2 gout flares in the past 6 months. Hmong ancestry was based on self-report of both parents being of Hmong descent. Individuals with any of the following were excluded: active liver disease, eCrCl <30mL/min, pregnant women or women of a child bearing age not using any form of contraception, inability to discontinue any ULT for 7-days, or history of allergic reactions to allopurinol.

This study was designed as a 3-visit study, including recruitment (Visit1/V1), pre-allopurinol (Visit2/V2), post-allopurinol (Visit3/V3) [Figures 5.1-2]. Halfway through the study, a salivary DNA-based recruitment (Visit0/V0) was added to expedite enrollment of participants with the less prevalent (TT) genotype for the rs505802 T>C. Hmong adults responding to either media advertisements or a personal encounter with board members came to either V0 or V1. After obtaining consent and completing an initial screening survey, salivary DNA was collected and processed to identify individuals with genotypes

of interest. At V1, weight, height, waist circumference, heart rate, blood pressure, a brief medical and medication history, and blood samples (for SUA level and basic blood biochemistry) were collected.

Assessment of UA disposition parameters including partial UA renal clearance ($CL_{R(UA)0\text{-}6hr}$) , urinary UA excretion ($UUE_{0\text{-}6hr}$) rate and percent UA fractional excretion ($FEUA_{0\text{-}6hr}$ %) over 6-hours was conducted before and after taking allopurinol.

At the beginning of V2, following a 10-hour overnight fast, venous blood was collected at 0 and 6-hours and urine was collected at 0, 2, 4, 6 hours. At the end of V2, participants were instructed to begin 100mg allopurinol every 12 hours for 7 days followed by 150mg allopurinol every 12 hours for 7 days. During V3, an assessment of oxipurinol and UA disposition parameters post-allopurinol conducted. Following a 10-hour overnight fast, venous blood was collected at 0, 2, 4, 6 hours to determine oxipurinol partial area under the serum concentration-time curve ($AUC_{0\text{-}6hr}$). Concomitantly, urine was collected at 0, 2, 4, 6 hours to estimate UA disposition parameters. Following 0-hour blood draws at V2 and V3, participants received water and traditional Hmong food for breakfast and lunch. The overall study design overview summary is shown in Figure5.1.

Medication Adherence:

All participants received at least 2 phone calls reminding them to take their medications, inquiring about adverse effects, and reminding them to return all used and unused dose-packed allopurinol. At V3, participants answered questions about their study medication experience, including timing of the last dose and possible adverse effects. Medication adherence rates for the 2-week study drug were calculated as a percentage (number of doses taken divided by the total number of doses expected).

Estimated Creatinine Clearance:

Estimated serum creatinine clearance (eCrCl) was calculated by Cockcroft-Gault method: [(140-age) x (Ideal Body Weight in kg)/ (72 x Serum Creatinine)] x (0.85 if female). Adjusted body weight was used for overweight or obese people.

Uric Acid Disposition Parameters:

All UA parameters were determined over 6-hours. Absolute SUA change was calculated as the difference between SUA measured at the 6-hour time at both V2 and V3. Normalized UUE rate (mg/min/1.73m^2) was calculated using urine volumes collected at each time point multiplied by its UA concentration over the course of 0-6 hours divided by 1.73m^2. $FEUA_{0\text{-}6hr}$ (%) was expressed as the UA renal clearance relative to creatinine (Cr) renal clearance. This was calculated by using the total urine UA and Cr concentrations (mg/L) produced over 6-hours and the average concentrations (0 and 6hr) of SUA and Cr (mg/L). The formula used was as follows:

$$FEUA_{0\text{-}6\ hr} = \frac{Total\ 6\ hr\ [UA]\ in\ Urine\ (mg/L) * Average\ [Cr]\ in\ Serum\ (mg/dL)}{Total\ 6\ hr\ \ [Cr]\ in\ Urine\ (mg/L) * Average\ \ [UA]\ \ in\ Serum\ (mg/dL)} X\ 100.$$

$UA/Cr_{0\text{-}6\ hrs}$ was calculated by comparing the amount of UA and Cr excreted in urine over 6-hours. $CL_{R(UA)0\text{-}6hr}$ was calculated by measuring the amount of UA excreted in urine over the 6-hour time collection divided by the average SUA concentrations at hour 0 and 6. For classifying hyperuricemia, the previously published criteria utilizing FEUA% and UUE rates were used to classify an individual's hyperuricemia as either overproducers or underexcreters or combined.[256] However, participants not meeting any of those specific

published criteria, FEUA% and UUE rate of $\geq$ 5.5% and $\leq$25 mg/hr/1.73m^2 were classified as normouricemia.

DNA Extraction and Genotyping:

DNA was extracted from peripheral blood using an Autopure LS automated extraction instrument and Qiagen Puregene® (Germantown, MD) reagents. In select participants, DNA was extracted from saliva by the ethanol precipitation method and the prepIT.L2P reagent for the purification of genomic DNA from the ORAgene DISCOVER (OGR-500) collection kit (Ontario, Canada). Finally, all DNA samples were prepared, labeled, and analyzed for quality and quantity. Initial genotyping for rs505802 T>C was conducted by a TaqMan® Genotyping Master Mix Protocol using the Polymerase Chain Reaction (PCR) method (Applied Biosystems, Foster City, CA, USA). Following the DNA amplification, a Real-Time PCR allelic discrimination protocol was used for plate reads. Subsequent multiplex genotyping was confirmed using iPLEX Gold method. iPLEX reagents and protocols for multiplex PCR, single base primer extension and generation of mass spectra were used as per the manufacturer's instructions. (iPLEX Application, Agena, San Diego, CA).

Uric Acid Assay:

Serum and urine UA concentrations were measured using a Roche COBAS 6000 chemistry analyzer (Roche Diagnostics, Indianapolis, IN) using the enzymatic method with a limit detection of 0.2mg/dL. The inter-assay coefficient of variation was 1.3% at 5.50mg/dL and 2.0 % at 9.67 mg/dL.

Serum Oxipurinol Assay:

The aliquoted serum samples were stored at -80°C until quantification within the Clinical Pharmacology Analytical Services Laboratory (Minneapolis, MN). Detection and quantification of oxipurinol in serum was performed using a high-performance liquid chromatograph (Agilent 1200 Series, Santa Clara CA) coupled with a TSQ Quantum triple stage quadrupole mass spectrometer (Thermo-Electron, San Jose, CA). The assay was linear in the range of 50 – 25000 mg/L of oxipurinol with accuracy and total variability of 98% and 3.9%, respectively. Details of the assay and method of detection have been previously published.[257]

Serum Oxipurinol Exposure Calculation:

Serum oxipurinol concentrations from 0 to 6-hours following the last dose were analyzed from 2-hour sampling times using non-compartmental analyses. $AUC_{0\text{-}6hr}$ was calculated from 4 time points (0, 2, 4, 6hr) using nominal time and log linear trapezoidal method by Phoenix® WinNonlin® 6.4 software (Pharsight, Certara L.P.)

Statistical Analysis:

A sample size of 30 individuals for rs505802 T>C within *SLC22A12* [13 (CC), 12 (CT), 5 (TT)] was estimated to achieve 80% power to detect an effect size of 1 between mean oxipurinol AUCs across 3 genotypes using an F-test with a significance level of 0.05. Accounting for a 20% drop out rate and using previously published estimates of oxipurinol AUC[169] and allele frequencies within the Hmong,[79] we estimated the need to recruit thirty-six participants.

Continuous data variables are expressed as mean (±SD) [range] and categorical data as percentages. Baseline and treatment values (pre- and post-allopurinol) were

compared using paired *t*-test. For multiple testing, one-way ANOVA was used followed by Bonferroni *t*-test adjustment for *post-hoc,* if significant. A stepwise multiple regression using an additive model with $F \geq 4.0$ for inclusion and $F \leq 3.9$ for exclusion was used to identify which selected SNPs [Table 5.7] were associated with the absolute change in SUA. The base model included baseline V-2 SUA, oxipurinol $AUC_{0\text{-}6hr}$, and V3 eCrCl. Shapiro-Wilk and Leven's tests were used to assess normality and homoscedasticity assumptions of the linear model, with $p<0.05$ to reject the null hypothesis.

Results

Table 5.1 summarizes the participant's demographics and characteristics. From November 2014 to December 2015, 36 study participants completed all phases of the GOUT-H study. Two participants were excluded from the final analyses due to poor (<79%) adherence to allopurinol. The mean (±SD) [range] adherence rate was 95% (±6) [79-100] for the 34 subjects included in the final analysis. Study participants were predominantly males 91% and 77% of participants were born outside the US. The mean (±SD) [range] age was 43.5 (±12.7) [24-67] years with 38% (13/34) <40 years of age.

Effect of Allopurinol on Uric Acid Disposition Parameters:

Table 5.3 summarizes the overall effects of allopurinol on UA disposition parameters. Allopurinol reduced the mean $UUE_{0\text{-}6hr}$ rate from 0.38 to 0.20 mg/min/1.73m^2 ($p<0.001$) [Figure 5.8], $CL_{R(UA)0\text{-}6hr}$ from 7.10 to 6.15 mL/min ($p=0.042$), $FEUA_{0\text{-}6hr}$% from 6.49 to 4.88% ($p<0.001$) [Figure 5.9], urine UA/urine Cr ratio from 0.54 to 0.26 ($p<0.001$). Allopurinol increased urinary creatinine excretion (UCrE) rate from 0.79 to 0.82 mg/min/1.73m^2 ($p=0.419$). Analysis of UA parameter changes based on rs505802

T>C within *SLC22A12* alone did not identify a statistically significant association between $FEUA_{0\text{-}6hr}$% either at baseline or post-allopurinol therapy.

Effect of Allopurinol on SUA and Kidney Function

The primary outcome was the absolute change in SUA after two weeks of allopurinol therapy. The mean(±SD) SUA decreased from 9.3 (±1.5) to 5.3 (±1.1) mg/dL, ($p<0.001$) with mean (±SD) [range] absolute SUA reduction of 4.0 (±1.5) [1.4-7.1] mg/dL [Figure 5.4]. The mean percent reduction of SUA was 41% (±12) with a range of 23-66%. Additionally, 71% (24/34) of study participants achieved the target SUA <6mg/dL [Figure 5.5]. Using the weight modified Cockcroft-Gault equation, the mean (±SD) eCrCl increased from 72 (±26) at V2 to 76 (±26) mL/min at V3 with a mean percent increase of 5% (P=0.029) [Figure 5.10].

Hyperuricemia and Gout Classification:

Twenty-three of 34 participants (68%) had diagnosis of gout based on self-reported history of using allopurinol or febuxostat. The remaining participants were enrolled based on SUA ≥ 6 mg/dL. Using the published criteria for classifying hyperuricemia status,[256] 38% (13/34) had UA underexcretion, 32% (11/34) had normal UA disposition, 24 % (8/34) had UA overproduction and 6% (2/34) had combined UA underexcretion and overproduction.

Pharmacogenetics of Oxipurinol

Thirty-four study participants completed the study, but only 33 participants had known serum oxipurinol concentrations due to one participant's inconsistent timing of taking allopurinol. Table 5.5 summarizes the key oxipurinol PK findings. The Mean ± (SD) Oxipurinol $AUC_{0\text{-}6hr}$ was 85.9 ± (34.7) (mg*hr/L) with a range of 39.9-176.6

(mg*hr/L) [Figure 5.11]. The mean (±SD) serum 0-hour oxipurinol concentration (C_{0hr}) and oxipurinol concentration at the 6-hour were 12.6 (±6.2) mg/L and14.0 (±5.8) mg/L, respectively. Stratified by genotype of rs505802 T>C, the mean (±SD) SUA absolute change were 4.21 (±1.6) mg/dL, 3.94 (±1.2) and 3.04 (±1.0) mg/dL, for the CC, CT and TT, respectively (p=0.29) [Figure 5.6,14]. The mean oxipurinol C_{0hr}, C_{6hr} and AUC_{0-6hr} were associated with *SLC22A12* genotype (p<0.001) [Figure 5.15-16]. Relative to the CC genotype, the AUC_{0-6hr} and C_{0hr} were lower for CT and TT genotypes (p<0.05). Although the study was purposely powered for analysis of a single SNP rs505802, analysis of contributions from 15 SNPs across 12 genes associated with baseline SUA or gout and allopurinol metabolism,[80,81] was conducted [Tables 5.8-10]. A base model consisting of baseline SUA, oxipurinol AUC_{0-6hr} and eCrCl explained 64% of the variability in absolute SUA reduction. Considering 15 SNPS and using a forward stepwise multivariate analysis, only rs505802 T>C within *SLC22A12* was found to be significantly (P=0.013) associated with absolute SUA reduction in addition to the base model [Table 5.6]. The final model explained 71% of the variability in absolute SUA reduction [Tables 5.7].

Allopurinol Therapy Adverse events:

Adverse drug events reported included gout flare-ups (11/34), drowsiness (3/34) and nausea (2/23). One participant had urinary retention that appeared to resolve by reducing allopurinol dose to 250mg/day. One participant experienced drowsiness and remained on allopurinol 100mg every 12 hours. One case of a mild rash resolved by taking an antihistamine and was discontinued from the study. Blood biochemistry measurements taken pre-and post-allopurinol indicated no clinically important changes.

Discussion

This is the first report to prospectively ascertain the impact of genetic factors on the pharmacokinetics and pharmacodynamics of allopurinol. Documenting a 41% SUA reduction facilitating 71% of participants to achieve target SUA levels < 6mg/dL with minimal adverse effects, provides evidence of overall efficacy and tolerability of allopurinol in the Hmong. Further analysis of measures of response to allopurinol based on rs505802 T>C within *SLC22A12* suggest this SNP contributes significantly to the variability in urate-lowering response in the Hmong. The significance of identifying rs505802 T>C affecting drug response is several-fold. Not only does the risk allele (C) for rs505802T>C, occur more frequently in the Hmong relative to non-Hmong,[79] the same allele has been significantly associated with baseline SUA and metabolic syndrome.[80,81,258]

The physiological and biological effects of genetic variants within *SLC22A12* on UA and oxipurinol are compelling. The URAT1 transporter, encoded by *SLC22A12*, is responsible for reabsorbing up to 90% of the filtered UA back into circulation.[182] In addition, evidence from *in vitro* data implicating the role of URAT1 in reabsorbing oxipurinol,[253] strongly suggests that a change in either the level of activity or expression of transporter would be expected to influence the amount of UA and oxipurinol reabsorbed. The predominance of the C allele in the Hmong (65%) relative to CEU (27%) ($p<0.001$),[79] adds to the possible genetic basis for the Hmong's documented predisposition to hyperuricemia and gout.[26,79] This alignment of observations support the hypothesis that genetic variation within URAT1 gene not only play a significant role in the prevalence of hyperuricemia and gout in the Hmong,[17] but also drug response through modulating

oxipurinol exposure.[253] Collectively, these observations support the hypothesis that rs505802 T>C within *SLC22A12* plays a significant role in the pharmacokinetics-pharmacodynamics of allopurinol.

The impact of rs505802T>C within *SLC22A12* on allopurinol's pharmacokinetics suggests that variability in response to allopurinol may partially be attributed to an individual's functionality of URAT1. From the present study, participants with the CC genotype had the highest oxipurinol $AUC_{0\text{-}6hr}$ and lowest oxipurinol renal clearance, resulting in a greater absolute SUA reduction compared with CT and TT genotypes [Figure 5.17]. This is consistent with the knowledge of the T allele (rs505802T>C) being significantly associated with lower SUA baseline and potentially lower oxipurinol exposure.[80,253] Moreover, the study identifies an inverse correlation between oxipurinol $AUC_{0\text{-}6hr}$ and eCrCl ($r=-0.64$, $p<0.001$) [Figure 5.12] and a positive correlation between oxipurinol $AUC_{0\text{-}6hr}$ and absolute SUA reduction ($r=0.59$, $P<0.001$) [Figure 5.13] confirming the importance of kidney function and oxipurinol exposure as significant determinants of response to allopurinol.[255]

There are other genetic factors affecting the development of gout and responsiveness to allopurinol. For example, previous reports retrospectively identified the rs2231142G>T within *ABCG2,* as a predictor of poor response to allopurinol.[86,165] The mechanism by which this SNP affects the response to allopurinol remains elusive and underscores the need for a mechanistic-based study to examine its paradoxical effect on the response to allopurinol.[86] In the present study, prospective enrollment based on rs505802T>C within *SLC22A12* did not yield a significant difference in the risk allele

frequency of rs2231142G>T relative to the expected frequency reported in a distinct cohort of Hmong[Table 5.8-9].[79] This observation suggests URAT1 could play a more significant role than ABCG2, at least within the Hmong sample studied. Furthermore, in contrast to rs2231142G>T within the *ABCG2*, analysis focusing on rs505802 T>C within *SLC22A12* provides a plausible mechanism for the variabilities in the pharmacokinetics and Pharmacodynamics of allopurinol.

Measuring oxipurinol concentrations have been proposed as a tool to assess compliance and guide allopurinol dosing.[163,255] For example, Emmerson et al[254] suggested that 6-9 hour post-allopurinol measurements of serum oxipurinol concentrations of 15.2 mg/L (100 µmol/L) are needed to achieve target SUA concentrations $\leq$ 7.0mg/dL (0.42 mmol/L). Stamp et al [255] have suggested 6-9 hours post-dose of 15.2-22.8 mg/L or trough oxipurinol levels of 10.0-15.2mg/L are needed to achieve the target SUA concentration < 6mg/dL.[255] In this study, the mean (±SD) 6-hour post-allopurinol and trough serum concentrations of oxipurinol were 14.0 (±5.8) mg/L and 12.6 (±6.2) mg/L, respectively suggesting that most individuals in the present study would be expected to achieve optimal SUA targets. However, summarizing mean data from the population ignores important sources of variability in oxipurinol such as allopurinol dose, renal function and genotype. Specifically, with 41% SUA reduction and 71% of participants achieving SUA < 6mg/dL in the present study, these outcomes compare favorably relative to other published clinical trials achieving 33-34% SUA reduction[259,160] with 45% of patients achieving SUA target with comparable dosing.[255] Nonetheless, we recognize that this study benefited from a greater adherence to allopurinol, in part due to its comparatively short duration, relative to

others.[255,259,160] However, participants achieving serum oxipurinol concentrations of < 15.2 mg/L at 6-hours occurred in 22/33 of study participants while trough concentrations of serum oxipurinol of <10mg/dL occurred in 13/33 of study participants. Stratified by, participants with the CC genotype had more than 2-fold higher oxipurinol $C_{0hr,}$ C_{6hr} and $AUC_{0\text{-}6hr}$ compared to the participants with the TT genotype [Table 5.5] [Figure 5.15-16]. Consequently, analysis of individuals failing to achieve target oxipurinol trough and 6-hours had 36% and 37% SUA reduction resulting in 54% and 55% achieving target SUA <6mg/L, respectively. Thus, understanding that genotype and population specific variability in allele frequencies may play a significant role in achieving target serum concentrations of oxipurinol underscores the importance of utilizing genetic knowledge in the context of individually guiding effective allopurinol dosage selection. Given that severe cutaneous reactions associated with allopurinol use is both dose and exposure dependent,[260,261] targeting SUA levels with the benefit of knowledge of an individual's genotype may provide additional rational for optimal dosage selection for allopurinol. The consistency of our observations with the proposed mechanistic role of rs505802 T>C within *SLC22A12* both oxipurinol concentrations and overall response to allopurinol provide additional support for further studies to validate and assess the potential value of genetic guidance when prescribing allopurinol.

Allopurinol is both a xanthine oxidase substrate and inhibitor. The changes in UA disposition parameters observed in the present study would be predictable based on this mechanism of action. Specifically, from Table 5.3, allopurinol reduced UA production without affecting its elimination. In general, most (90%) individuals with gout are

classified as UA underexcreters.[70] In the present study, however, only 38% of study participants met the definition of UA underexcreters. This observation raises questions about possible differences between populations in terms of the physiological basis for the etiology of and therefore possible differences in approaches to manage it.

One significant barrier to using allopurinol in the Hmong relates to their perceptions of allopurinol's ineffectiveness and/or perceived renal toxicity. Failure to overcome skepticism of Western medicine and unjust perceptions of toxicities may adversely affect medication adherence to allopurinol.[26] Our findings of a mean increase in eCrCl of 5% (p=0.029) was consistent with the effect of long-term use of allopurinol on kidney function.[262] Though it was a small improvement, it goes a long way toward addressing the unjust perceptions of allopurinol toxicity.

In summary, the rs505802T>C within *SLC22A12* significantly affects the disposition of oxipurinol and response to allopurinol in the Hmong. These findings of genetic basis to variability in response to allopurinol within the Hmong may translate into opportunities to address health disparities in prevalence and treatment of gout in the Hmong, while advancing precision medicine to a broader array of members of our society. Although validation of our findings is required, this trial demonstrated the role and value of forming a community partnership to uncover significant findings of genetic polymorphisms which modulate response to allopurinol. Once validated, such genetic findings may eventually form the basis for guidance of clinical decision tools to help manage Hmong and non-Hmong patients with gout.

Limitations

Several limitations require acknowledgement. Although both men and women were recruited, only 3 women participated in this study. Although this limits the generalizability of our findings, it is not unexpected since gout is more prevalent in men than women and specifically Hmong men.[17] As a pilot study to test the genetic basis of response to allopurinol, the study duration and sample collections were naturally limited.

A short study duration precludes an evaluation of the long-term clinical outcomes of allopurinol therapy by genotype, yet was adequate to establish the effect of rs505802T>C within *SLC22A12* on the pharmacokinetics and pharmacodynamics of allopurinol. Limitations which are common to studies of special populations, included our 6-hour sampling scheme for blood and urine collection. Based on other pharmacokinetics studies, we believe that the 6-hour window provides adequate measurement of data for our analysis of the principle endpoint of oxipurinol exposure by genotype while recognizing and respecting the guidance provided by the Hmong genomics board.

Although we specifically designed this study to focus on rs505802T>C within *SLC22A12*, analysis of the majority of select allele frequencies in the study did not significantly differ from what is expected in the Hmong population at large.[79] Finally, while participants were encouraged to maintain a consistent diet and medication regiments during the study, we could not account for any dietary changes which may have occurred while taking allopurinol. Notably, two participants reported the use of indomethacin and losartan, which may affect UA disposition, however, their use of these medications were confirmed to be consistent.

Figure 5:1 GOUT-H Study Design Overview

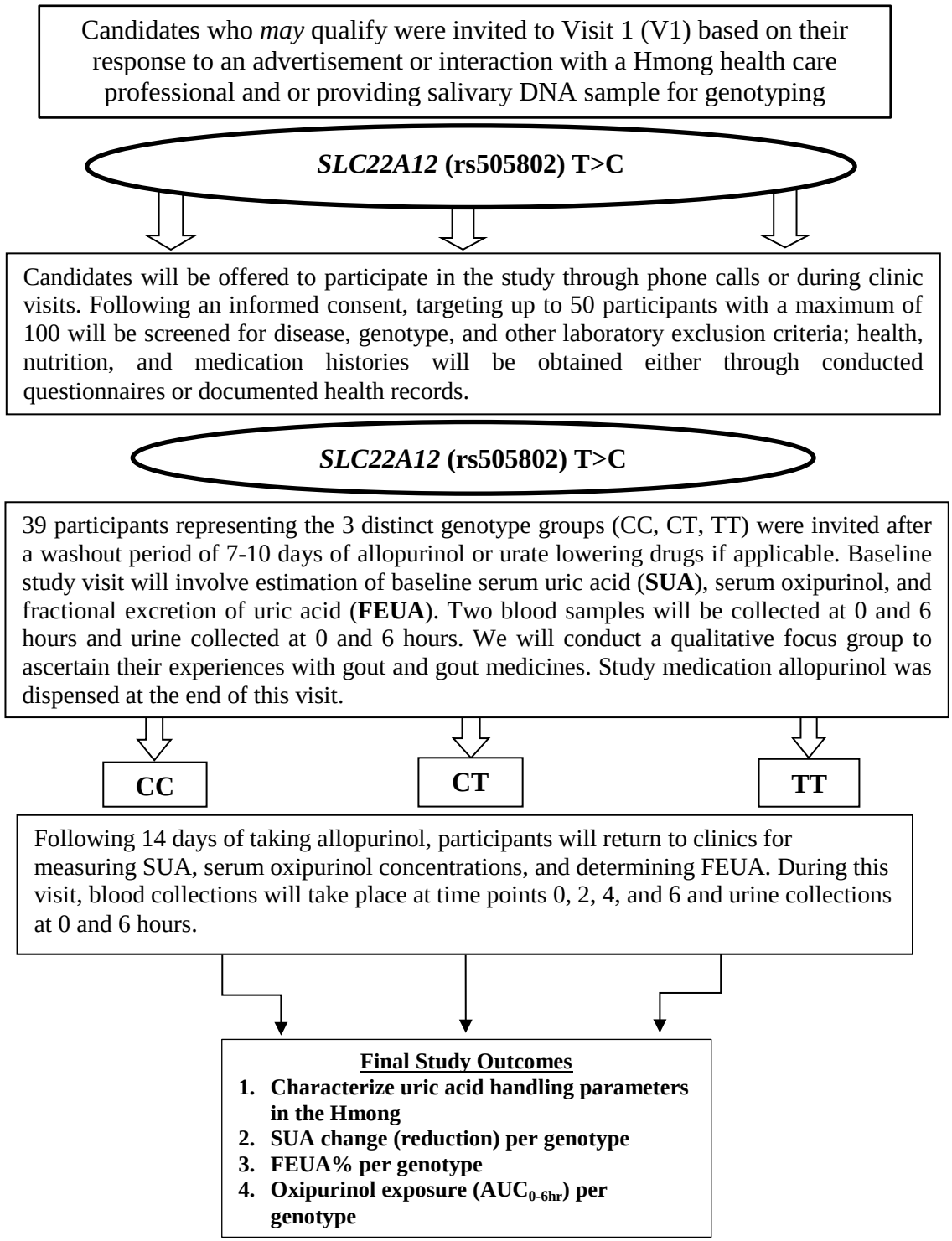


Figure 5:2 GOUT-H Flow Chart of Study Participants Enrollment

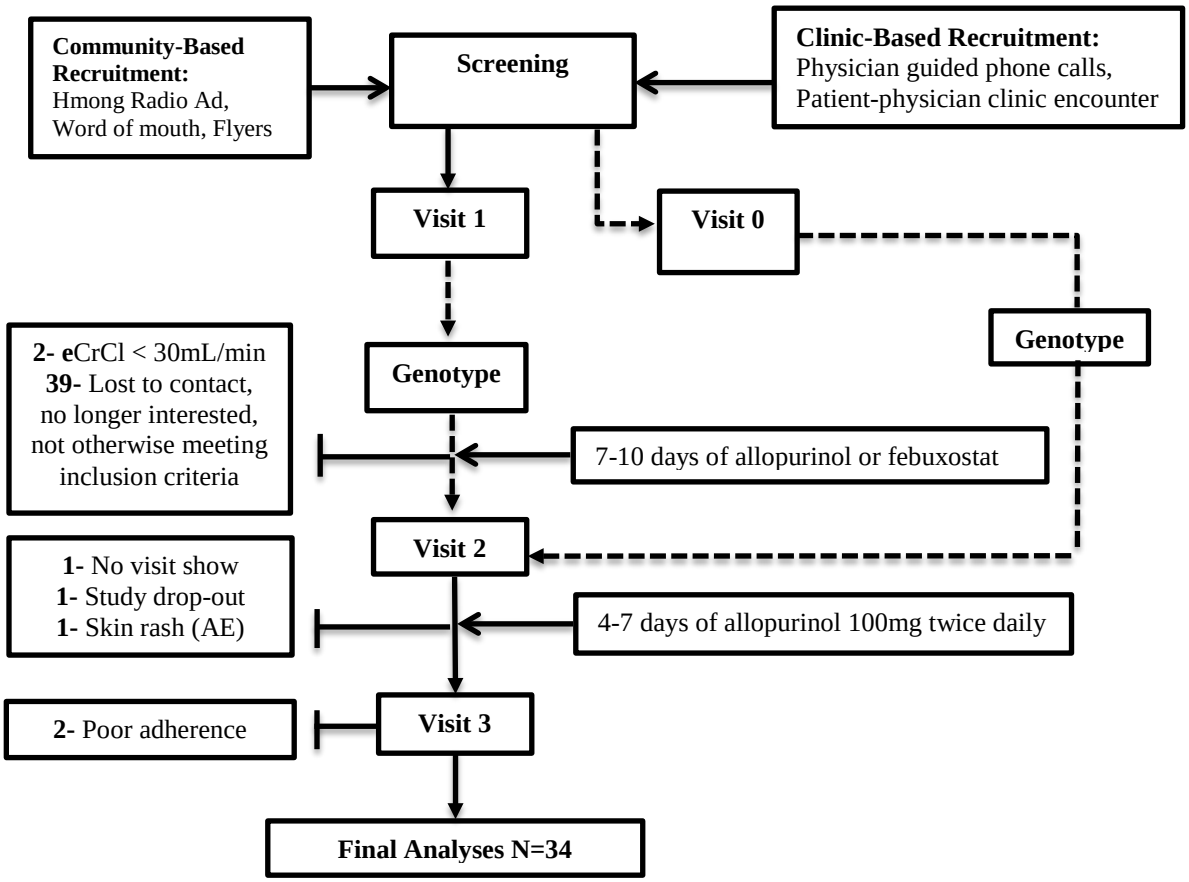


Figure 5:3 Box plots of serum uric acid levels at enrollment (V1), pre-allopurinol (V2) and post-allopurinol (V3) at both 0hr and 6hr

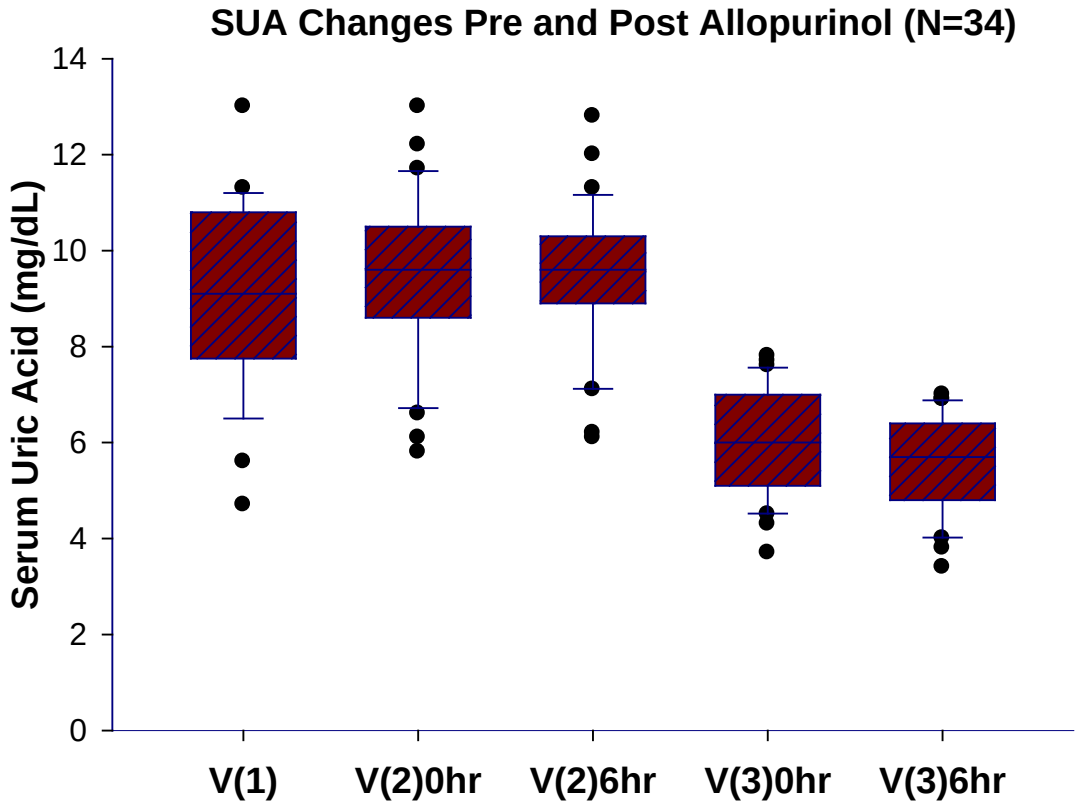


Figure 5:4 Mean serum uric acid levels pre-and post-allopurinol

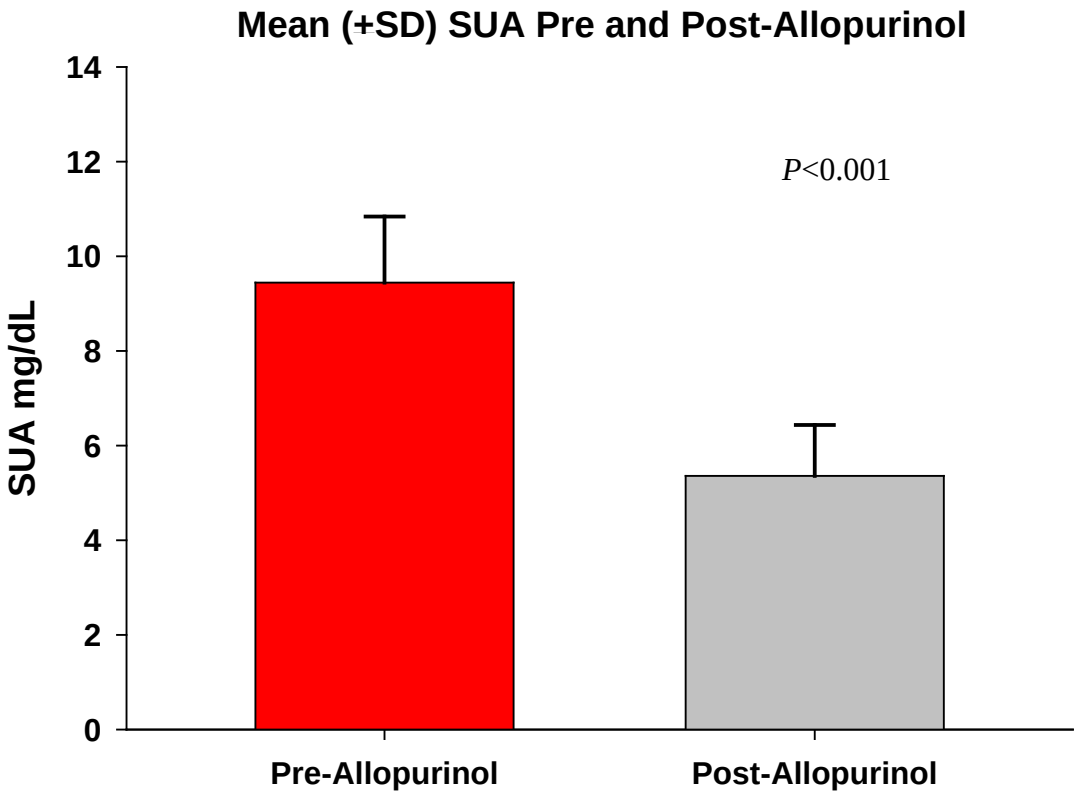


Figure 5:5 Serum uric acid pre-and post-allopurinol for all study participants

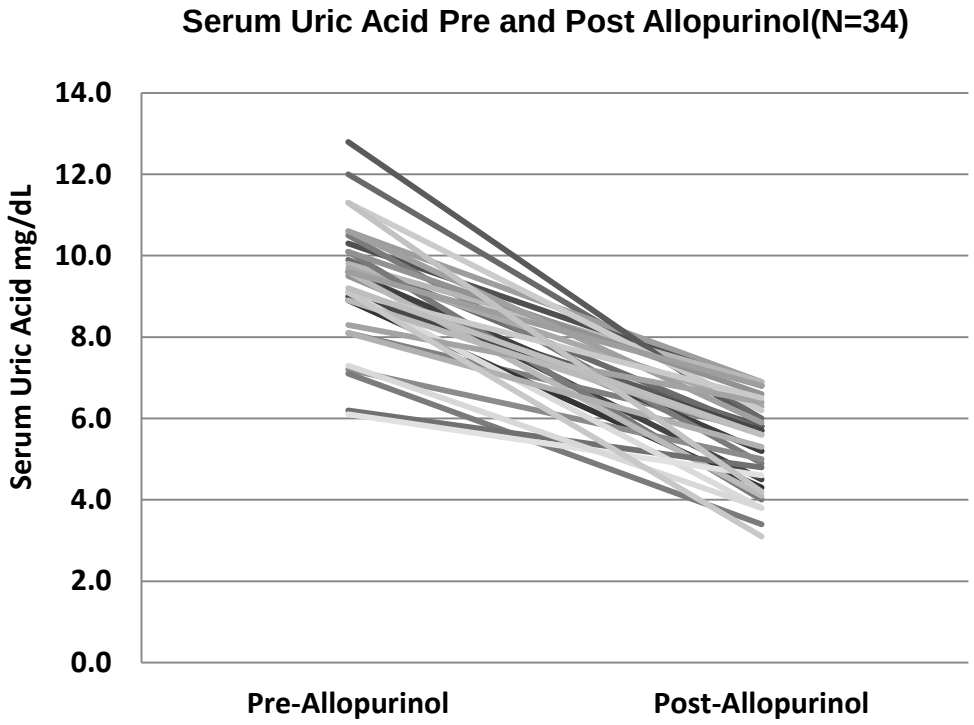


Figure 5:6 Serum uric acid pre-and post-allopurinol by genotype of rs505802 T>C

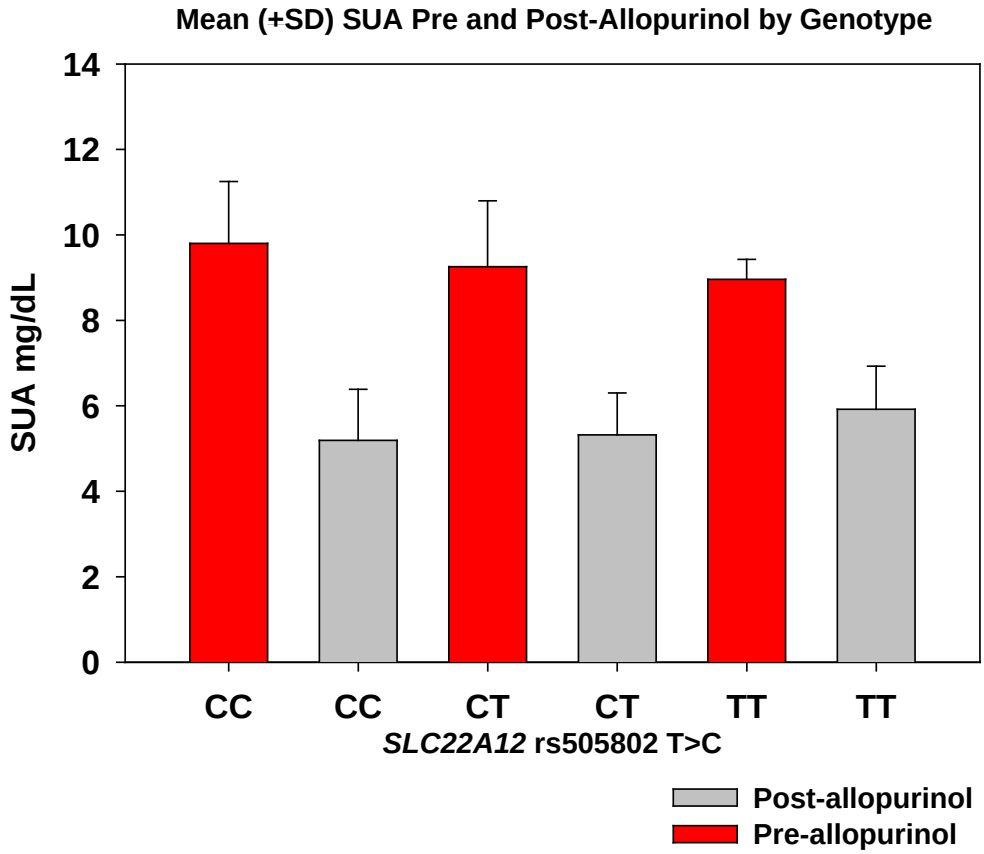


Figure 5:7 Mean absolute change in serum uric acid by genotype of rs505802T>C

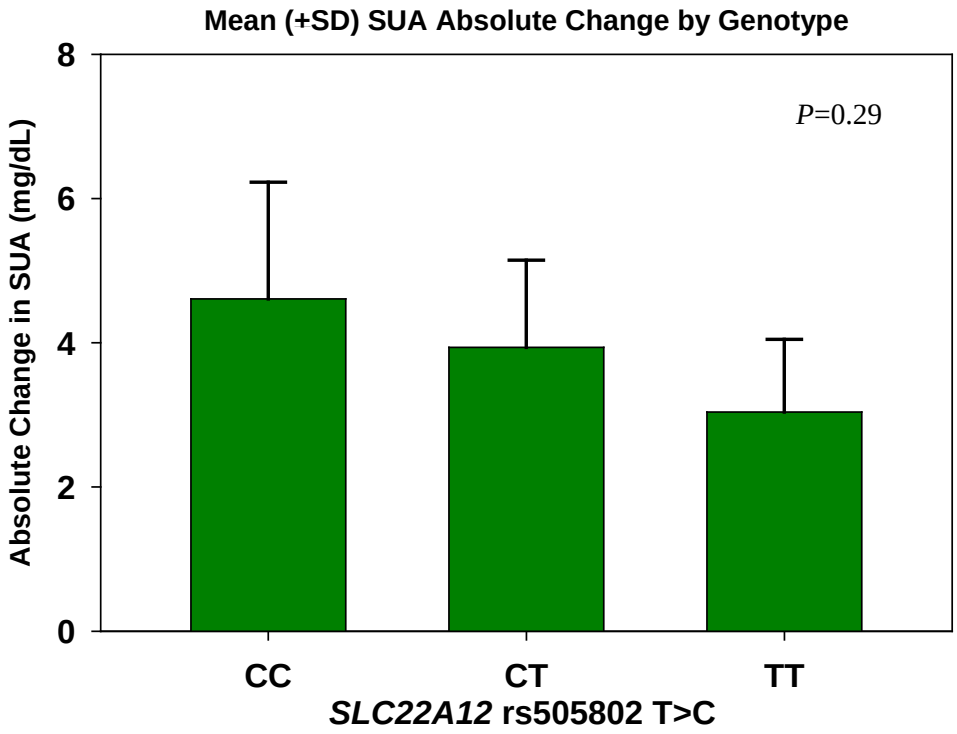
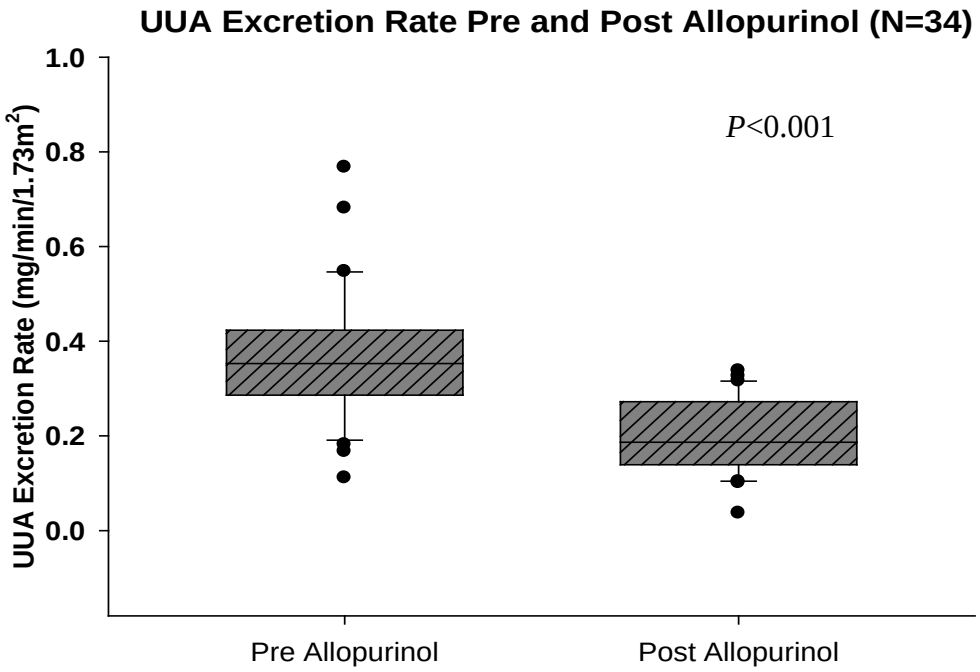
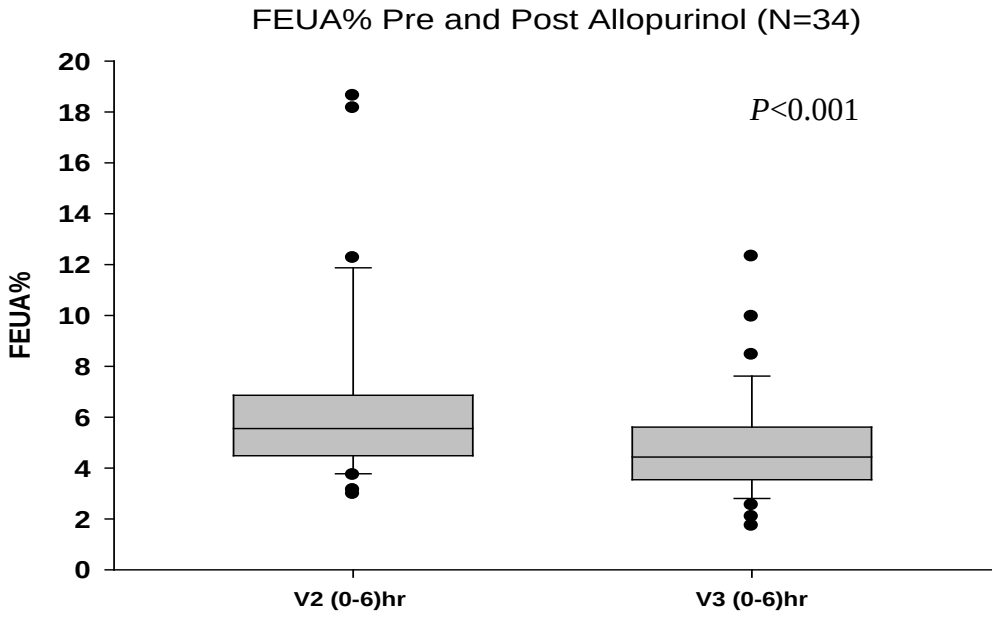


Figure 5:8 UUA Excretion Rate Pre and Post Allopurinol



UUA: Urinary Uric Acid

Figure 5:9 FEUA% Pre and Post-allopurinol



FEUA: Fractional Excretion of Uric Acid

Figure 5:10 Box plots of estimated creatinine clearance (eCrCl) pre-allopurinol (V2) and post-allopurinol (V3) at 0hr using Cockcroft-Gault method

.

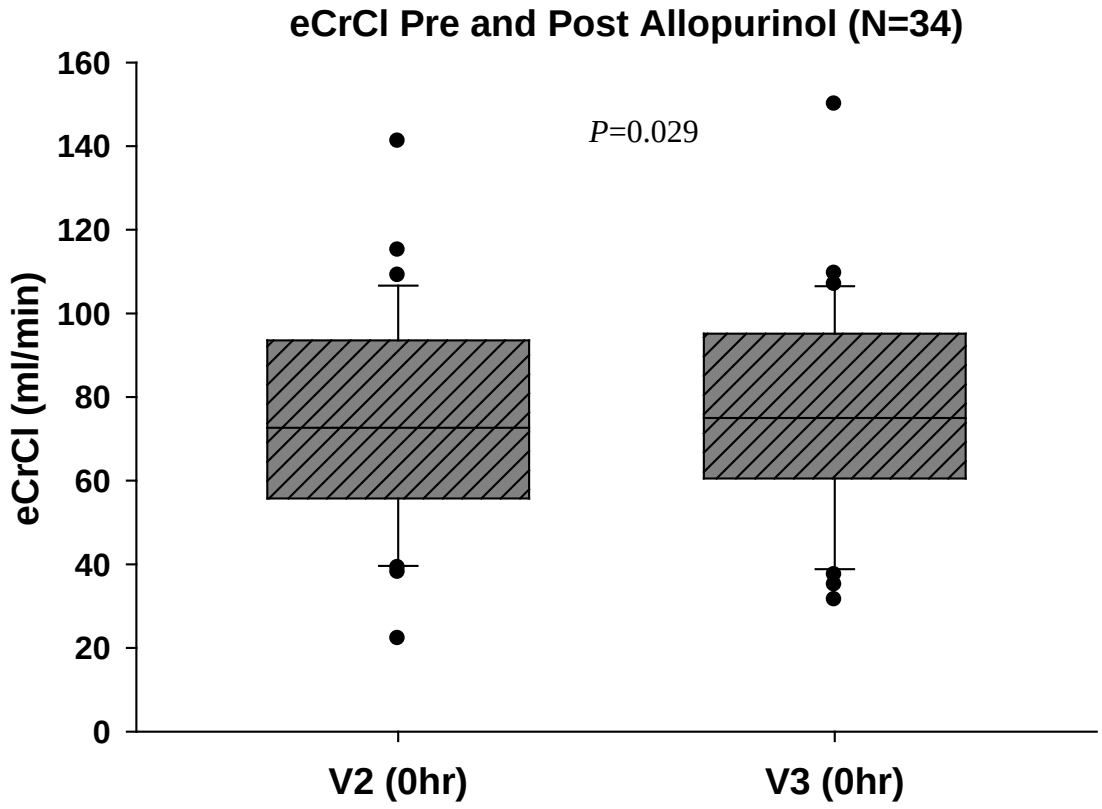


Figure 5:11 Oxipurinol area under concentration-time profile from 0 to 6 hours

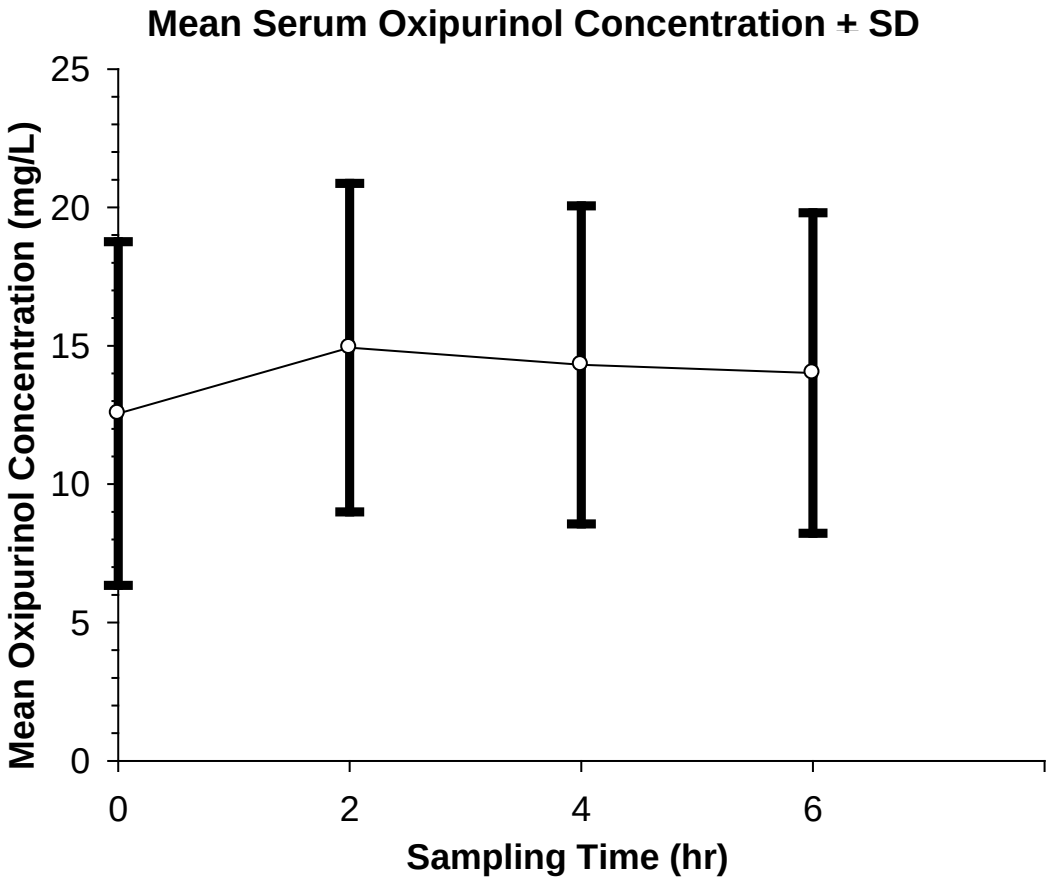


Figure 5:12 Correlation of oxipurinol AUC0-6hr with creatinine clearance at V3 using simple linear regression

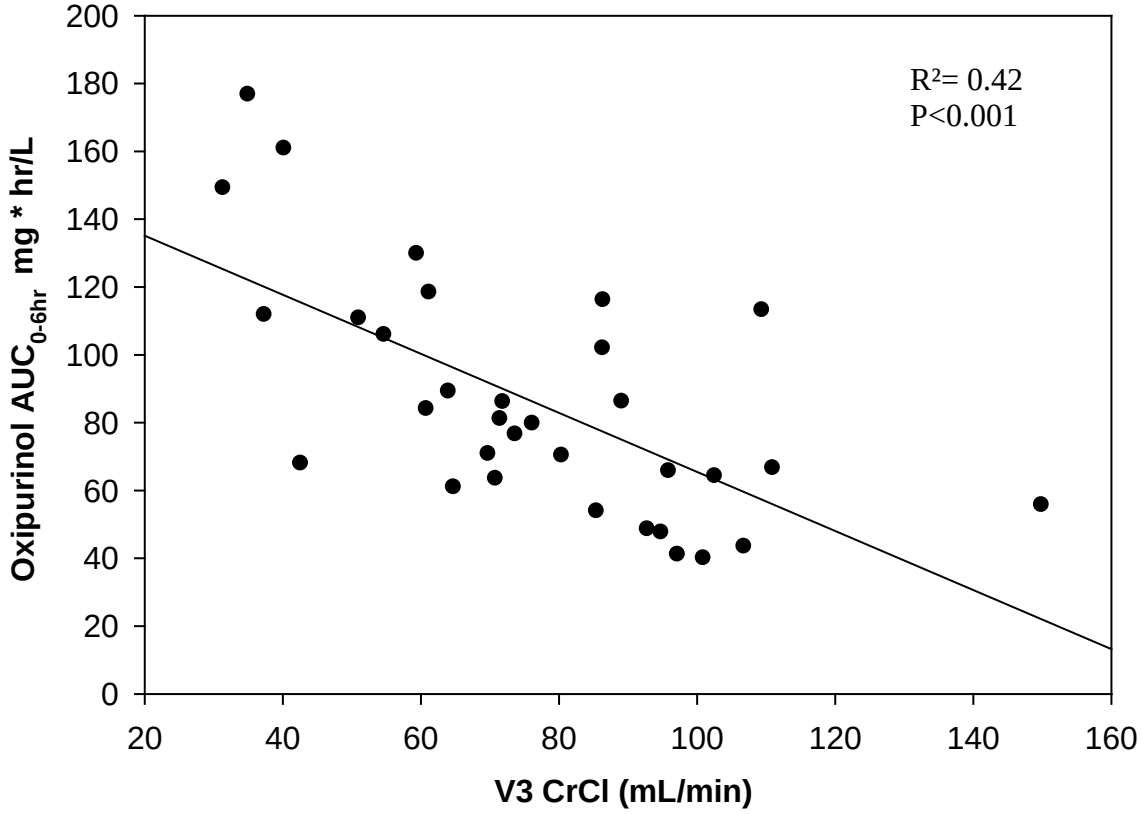


Figure 5:13 Correlation of absolute change in SUA (V2-V3) with 0xipurinol AUC0-6hr

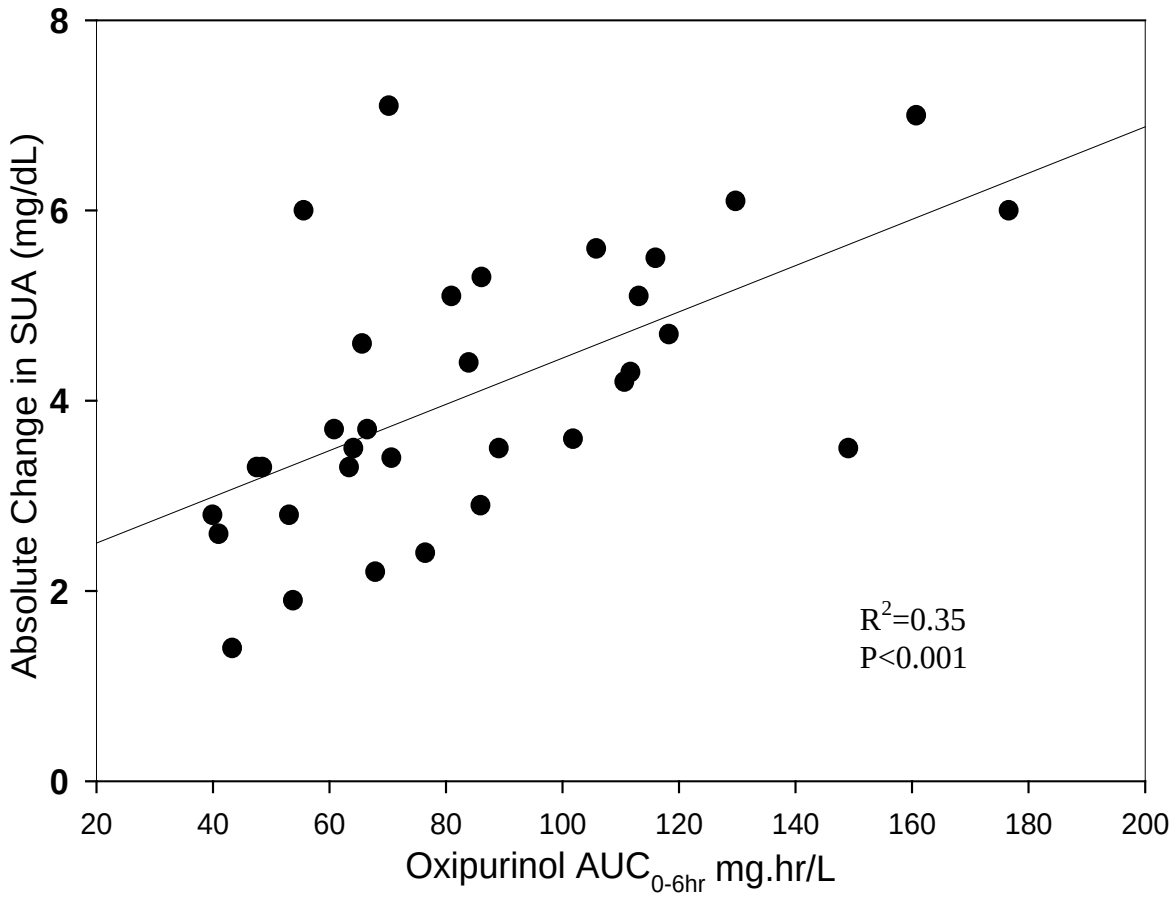


Figure 5:14 Scatter plots of absolute change in Serum uric acid (V2-V3) by genotype of rs505802T>C

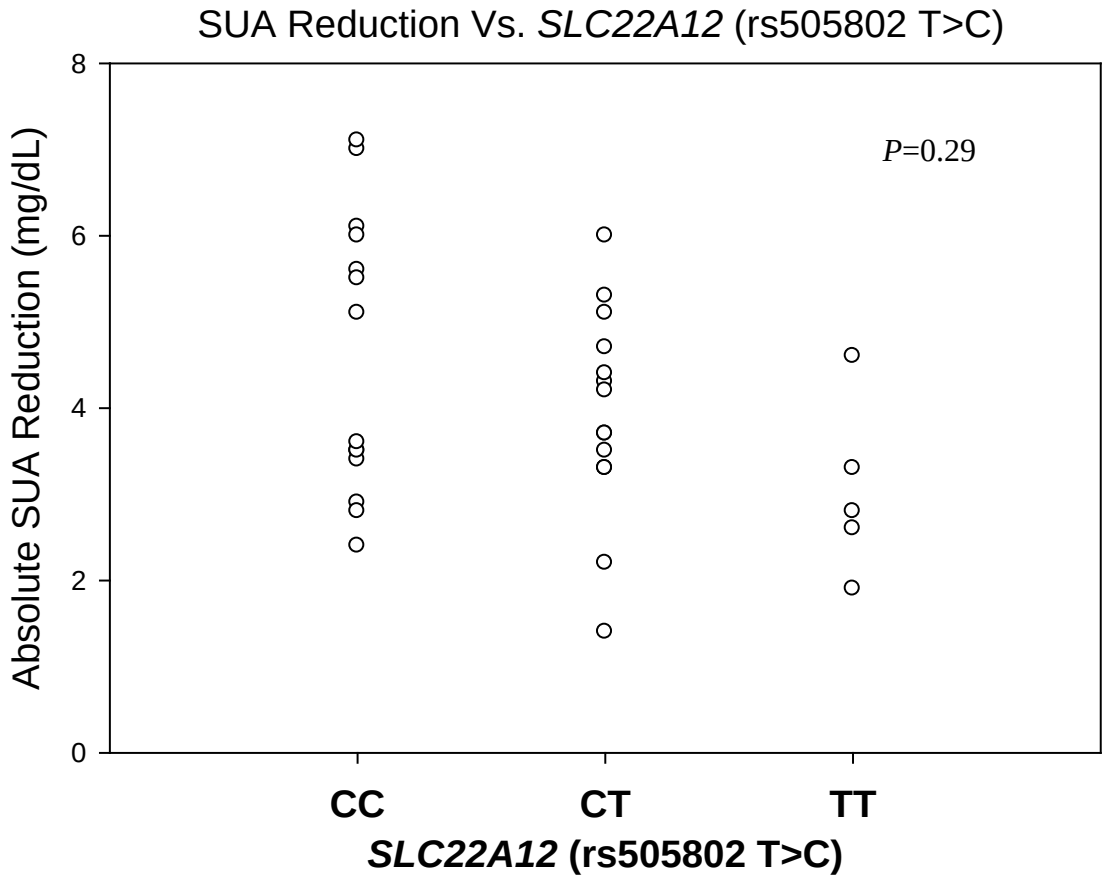


Figure 5:15 Scatter plot of oxipurinol AUC0-6hr by genotype of rs505802T>C

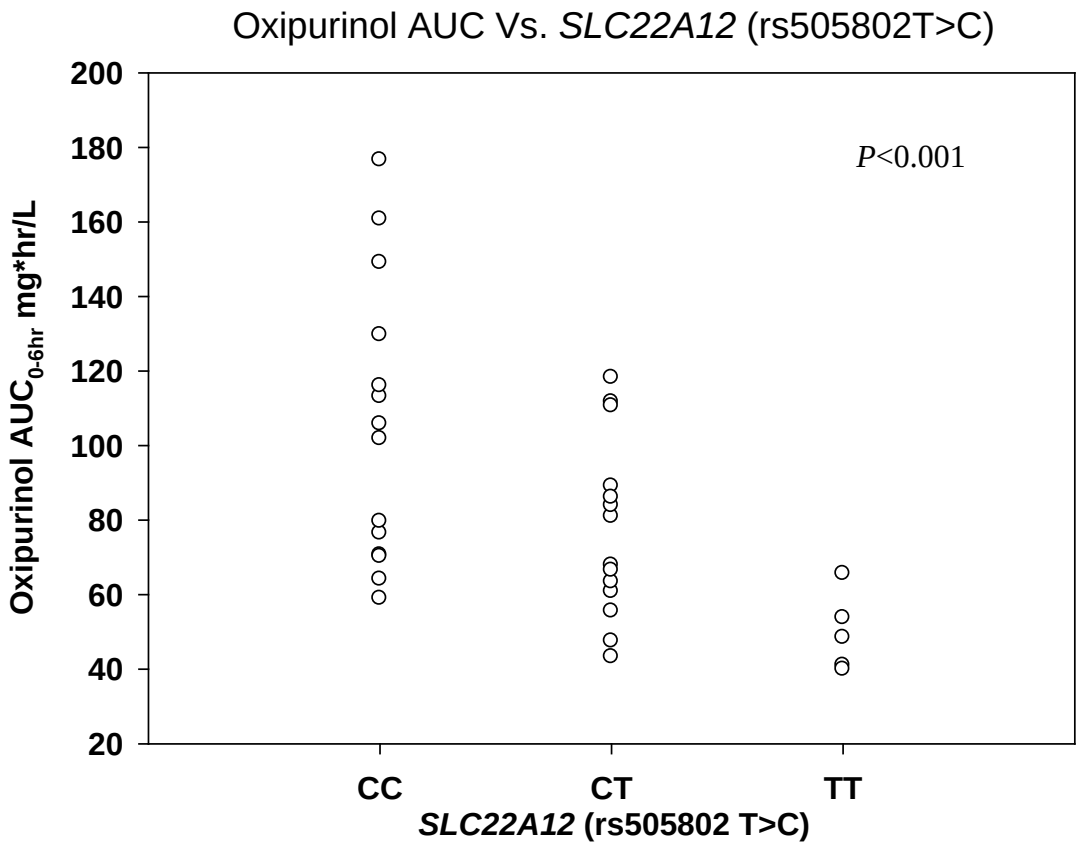
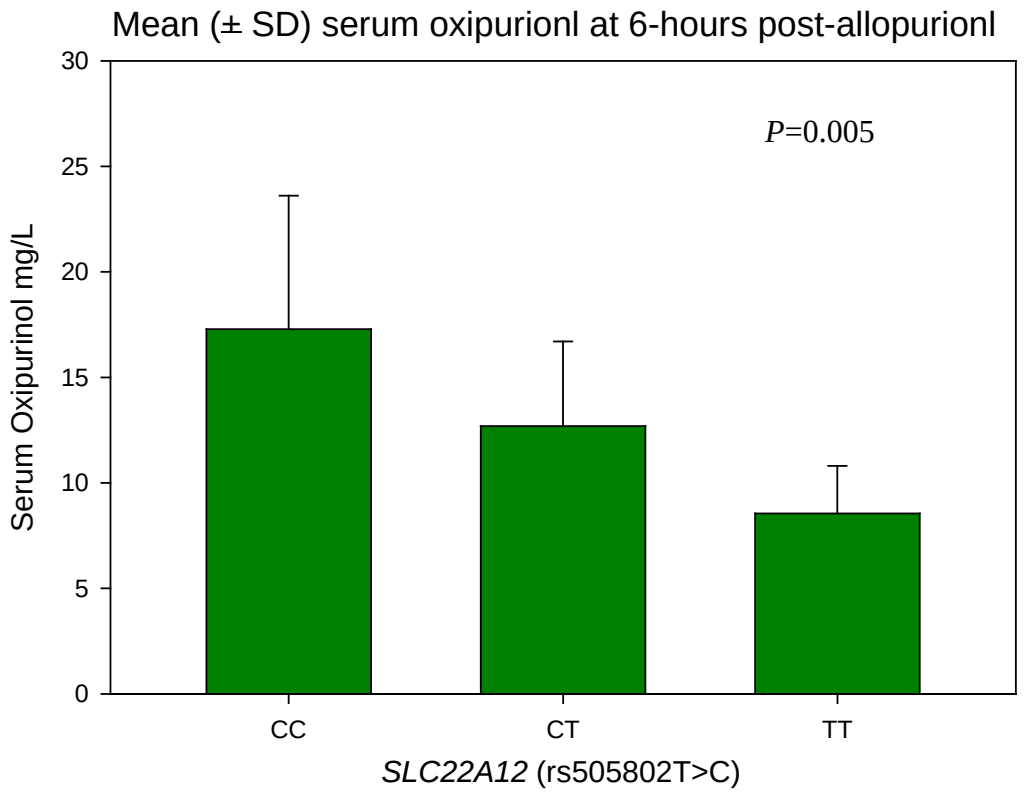
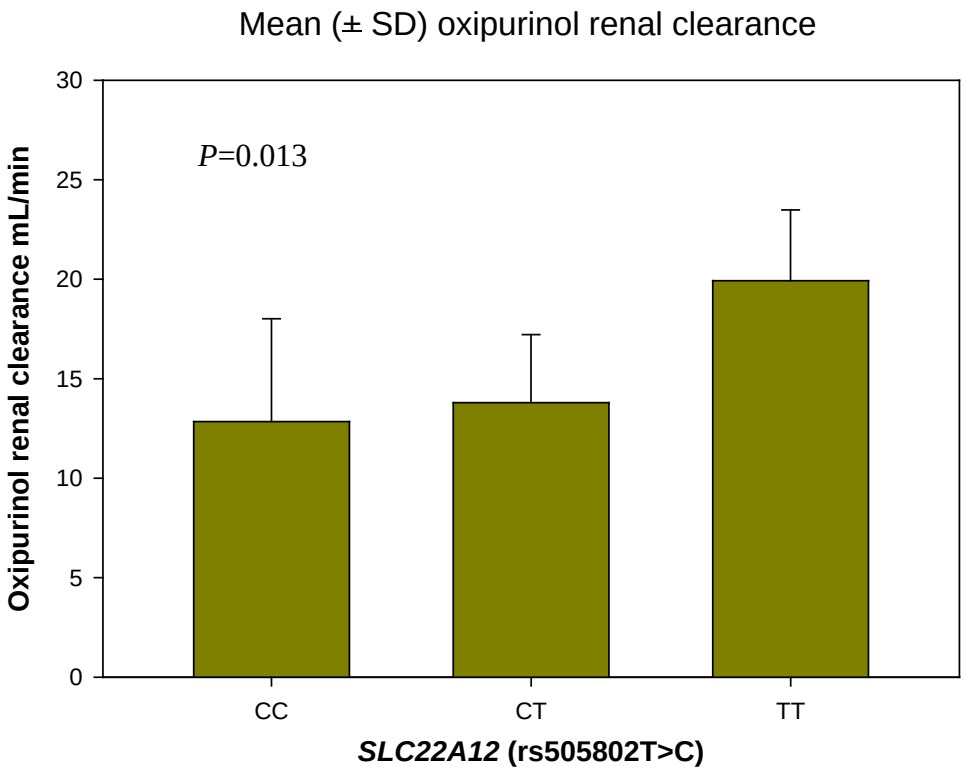


Figure 5:16 Mean (±SD) serum oxipurinol at 6-hour post allopurinol dose by genotype for rs505802.



Analysis was conducted using One-Way ANOVA with Bonferroni correction for Post-Hoc analysis with ($p<0.05$) for significance.

Figure 5:17 Mean (±SD) renal oxipurinol clearance by genotype for rs505802.



$$Oxipurinol\ Renal\ Clearance\ (mL/min) = \frac{Urine\ Oxipurinol\ (0-6)\ hr\ (mg)}{Serum\ Oxipurinol\ AUC\ (0-6)\ hr\ (mg.min/mL\ )}$$

Analysis was conducted using One-Way ANOVA with Bonferroni correction for Post-Hoc analysis with ($p<0.05$) for significance.

Table 5:1 GOUT-H Demographics Results Summary (N=34) at Visit 1			
Characteristics	N (%)	Mean (±SD)	Range
Gender			
o **Male**	31 (91%)		
o **Female**	3 (9%)		
Age (years)		43.5 (±12.7)	24 to 67
o **< 40**	13 (38%)		
o **≥ 40**	21 (62%)		
Country of Birth			
o **Laos**	24 (70.6%)		
o **Thailand**	2 (5.9%)		
o **US**	8 (23.5%)		
Highest Level of Education			
o **ESL**	6 (17.6%)		
o **High School**	4 (11.8%)		
o **College**	17 (50%)		
o **Graduate**	3 (8.8%)		
o **Missing**	4 (11.8%)		
English Proficiency			
o **Poor**	3 (8.8%)		
o **Fair**	6 (17.6%)		
o **Good**	14 (41.2%)		
o **Excellent**	7 (20.6%)		
o **Missing**	4 (11.8%)		
Immigration Year			1970 to 2009
o **1970-1979**	6 (23.1%)		
o **1980-1989**	8 (30.1%)		
o **1990-1999**	8 (30.1%)		
o **2000-2009**	3 (11.5%)		
o **Missing**	1 (3.8%)		
Years in the US		27 (±9.5)	6 to 45
Smoking History			
o **Never**	27 (79.4%)		
o **Quit**	4 (11.8%)		
o **Current**	3 (8.8%)		

Table 5.1 Continued			
Characteristics	N (%)	Mean (±SD)	Range
Height (in)		62.8 (±2.7)	57.5 to 68
o **< 60inches**	5 (15%)		
o **≥ 60 inches**	29 (85%)		
BMI (Kg/M^2)		32.5 (±5.4)	21.6 to 46.9
o **< 18.5**	0 (0%)		
o **18.5 – 24.9**	2 (6%)		
o **25 – 29.9**	7 (21%)		
o **30 – 39.9**	23 (68%)		
o **≥ 40**	2 (6%)		
Waist Circumference (inches)		40.6 (±5.1)	29.0 to 50.8
Males		40.2 (±5.2)	29.0 to 50.8
o **≤ 40**	15 (48%)		
o **> 40**	16 (52%)		
Females		44.5 (±1.9)	42.7 to 46.5
o **≤ 35**	0 (0%)		
o **> 35**	3 (100%)		
Heart Rate (bpm)		76 (±12)	54 to 99
Systolic Blood Pressure (mmHg)		141 (±17)	114 to 182
o **< 140**	19 (56%)		
o **≥ 140**	15 (44%)		
Diastolic Blood Pressure (mmHg)		91 (±12)	69 to 121
o **< 90**	18 (53%)		
o **≥ 90**	16 (47%)		
eCrCl (mL/min)		74 (±26)	39 to 148
o **eCrCL ≥ 60mL/min**	23 (65%) 11 (35%)		
o **eCrCl < 60mL/min**			
Self-reported health conditions			
o **Gout**	23 (68%)		
o **Kidney stone**	4 (12%)		
o **Diabetes**	7 (21%)		
o **Hypertension**	14 (41%)		
o **Hyperlipidemia**	13 (38%)		
o **Heart attack**	3 (9%)		
o **Stroke**	2 (6%)		
o **Others**	2 (6%)		

Table 5.1 Continued	
Participants reported medication use	
o XOI	19 (56%)
o Colchicine	8 (24%)
o NSAID	13 (38%)
o Opioid	10 (29%)
o Corticosteroids	9 (26%)
o ACE-I	7 (21%)
o ARBs	2 (6%)
o B-blockers	6 (18%)
o CCB	5 (15%)
o Diuretics	4 (12%)
o LLT	7 (21%)
o Antidiabetic	
o Non-insulin	9 (26%)
o Insulin	4 (12%)
o PPI	7 (21%)
o Sucralfate	1 (3%)
o Aspirin	7 (21)
o Anticoagulant	1 (3%)
o Vasodilator	2 (6%)
o Antidepressants	6 (18%)
o Hypothyroid therapy	1 (3%)
o Nitrate	1 (3%)
o H1RA	2 (6%)
o *Others	19 (56%)

XOI: Xanthine Oxidase Inhibitor
NSAID: Non-steroidal anti-inflammatory drug
ACE-I: Angiotensin converting enzyme inhibitor
ARBs: Angiotensin receptor blockers
CCB: Calcium channel blocker
LLT: Lipid lowering therapy
PPI: Proton pump inhibitor
H1RA: Histamine 1 receptor antagonist
*Predominantly supplements, non-western and non-FDA approved drugs

Table 5:2 Participants biochemical laboratory results			
Serum Biochemistry	**Baseline (N=34)** **Mean ± (SD)** **Median [range]**	**Pre-Allopurinol (N=34)** **Mean ± (SD)** **Median [range]**	**Post-Allopurinol (N=34)** **Mean ± (SD)** **Median [range]**
ALT (U/L)	31.62 ± 18.07 26.0 [13.0 – 89.0]		30.71 ± 14.78 26.0 [12.0 – 67.0]
AST (U/L)	24.79 ± 12.34 21.0 [13.0 – 63.0]		24.44 ± 8.89 22.50 [12.0 – 46.0]
BILT (mg/dL)	0.48 ± 0.24 0.4 [0.2 – 1.1]		0.46 ± 0.18 0.4 [0.2 – 1.0]
BUN (mg/dL)	17.15 ± 4.83 18.0 [9.0 – 28.0]		17.41 ± 5.67 16.0 [8.0 – 32.0]
GLU (mg/dL)	144.76 ± 99.30 100.5 [67.0-464.0]		116.59 ± 57.34 97.5 [69.0 – 320.0]
HCT (%)	42.27 ± 6.63 43.1 [11.6 – 49.6]		42.25 ± 3.76 42.45 [26.7 – 47.9]
HGB (g/dL)	14.54 ± 1.51 14.8 [9.1 – 16.6]		14.13 ± 1.52 14.25 [7.4 – 16.3]
PLT (x10e9/L)	228.97 ± 51.40 233.0 [68.0 –332.0]		232.62 ± 51.57 226.00 [159.0 – 385.0]
WBC (x10e9/L)	7.21 ± 2.00 7.65 [2.80 – 10.30]		8.63 ± 2.23* 8.65 [4.90 – 13.60]
K (mmol/L)	4.01 ± 0.36 4.10 [3.10 – 4.60]		4.17 ± 0.33 4.20 [3.50 – 4.80]
Na (mmol/L)	138.15 ± 2.81 139.0 [131.0 –143.0]		139.44 ± 2.58 139.0 [135.0 – 144.0]
SCR (mg/dL)	1.08 ± 0.24 1.03 [0.60 – 1.59]		
SCR (mg/dL) 0hr		1.10 ± 0.30 1.00 [0.70 – 2.30]	1.04 ± 0.24 0.99 [0.63 – 1.72]
SCR (mg/dL) 6hr		1.10 ± 0.3 1.0 [0.7 – 2.2]	1.06 ± 0.28 0.99 [0.62 – 1.94]
SUA (mg/dL)	9.05 ± 1.92 9.25 [4.70 – 13.0]		
SUA (mg/dL) 0hr		9.40 ± 1.70 9.70 [5.80 – 13.00]	5.81 ± 1.16* 5.95 [3.60 – 7.80]
SUA (mg/dL) 6hr		9.30 ± 1.50 9.60 [5.80 – 12.80]	5.32 ± 1.09* 5.60 [3.10 – 6.90]
eCrCl (mL/min)	74 ± 26	72 ± 26	76 ± 26*

* Indicates statistical significant ($p<0.05$) between V1 and V3 or V2 and V3

Table 5:3 Uric acid parameters pre-and post-allopurinol therapy (N=34)			
Parameter	Pre-Allopurinol (V2) (0-6) hr	Post-Allopurinol (V3) (0-6) hr	P-value
	Mean $\pm$ (SD	Mean $\pm$ (SD)	
UUE Rate (mg/min/1.73 m^2)	0.38 $\pm$ (0.14)	0.20 $\pm$ (0.08)	<0.0001
UCrE Rate (mg/min/1.73 m^2)	0.79 $\pm$ (0.22)	0.82 $\pm$ (0.26)	0.419
$CL_{R(UA)}$ (mL/min)	7.10 $\pm$ (2.61)	6.15 $\pm$ (2.04)	0.042
FEUA %	6.49 $\pm$ (3.60)	4.88 $\pm$ (2.14)	<0.0001
Urine Spot Ratio (U_{UA}/U_{Cr})	0.54 $\pm$ (0.22)	0.26 $\pm$ (0.13)	<0.0001

UUE= Urinary Uric Acid Excretion
UCrE= Urinary Creatinine Excretion
FEUA= Fractional Excretion of Uric Acid
$CL_{R(UA)}$= Uric Acid Renal Clearance
U_{UA}= Urine Uric Acid
U_{Cr}= Urine Creatinine

Table 5:4 Pharmacodynamics summary of allopurinol by genotype				
SLC22A12 (rs505802T>C)	**Count**	**Mean SUA (mg/dL) (±SD)**		
		Pre-allopurinol	**Post-allopurinol**	**Absolute Change**
CC	14	9.80 (±1.45)	5.19 (±1.19)	4.21 (±1.6)
CT	14	9.26 (±1.54)	5.32 (±0.98)	3.94 (±1.2)
TT	5	8.96 (±0.47)	5.92 (±1.01)	3.04 (±1.0)

Table 5:5 Pharmacodynamics summary of allopurinol by genotype				
***SLC22A12* (rs505802) Genotype**	**Mean ± (SD)**	**Range**	**95% CI**	***P*-value***
	Oxipurinol $AUC_{0\text{-}6hr}$ (mg*hr/L)			
CC (n=14) Reference	107.1± (36.0)	64.1-176.6	89.5 to 130.9	
CT (n=14)	77.5± (23.9)	43.3-118.2	63.7 to 91.3	0.032
TT (n=5)	49.7 ± (10.5)	39.9-65.6	36.6 to 62.8	0.002
	Oxipurinol C_{0hr} (mg/L)			
CC (n=14) Reference	15.7 ± (6.7)	5.8-28.0	11.8 to 19.6	
CT (n=14)	11.0 ± (3.9)	4.3-17.5	8.7 to 13.3	0.046
TT (n=5)	6.9 ± (1.7)	5.2-9.4	4.8 to 9.0	0.005
	Oxipurinol C_{6hr} (mg/L)			
CC (n=14) Reference	17.3 ± (6.3)	8.5- 28.4	13.7 to 20.9	
CT (n=14)	12.7 ± (4.0)	6.4-19.5	10.4 to 15.0	0.064
TT (=5)	8.5 ± (2.3)	6.7-12.3	5.6 to 11.4	0.006
	Oxipurinol Renal Clearance (mL/min)			
CC (n=14)	12.8 ± (5.2)	3.9-24.2	9.8 to 15.8	0.01
CT (n=14)	13.8 ± (3.6)	9.0-20.0	11.7 to 15.6	0.03
TT (n=5) Reference	19.9 ± (3.6)	15.9-23.6	15.4 to 24.4	

*One-Way ANOVA followed by Bonferroni t-test for *Post-hoc* analysis with $P<0.05$ for significance

Table 5:6 Stepwise multiple Linear Regression summary model 2				
Independent Variable	Beta Coefficient	*P*-value	R^2	Adjusted R^2
Constant	-4.828	0.001	0.71	0.67
V2 SUA_{6hr}	0.34	0.004		
Oxipurinol $AUC_{(0-6)\ hr}$	0.04	<0.001		
V3 eCrCl (mL/min)	0.021	0.009		
SLC22A12 (rs505802T>**C**)	0.68	0.013		

Note: SNP rs505802 was analyzed using an additive model. The rs505802 T>C was coded in the following manner: TT=0, CT=1 and CC=2

Table 5:7 Base-model and final model Summaries									
Model	R	R^2	Adjusted R^2	Std. Error of the Estimate	Change Statistics				
					R^2 Change	F Change	df1	df2	Sig. F Change
1	0.797[a]	0.636	0.596	0.8781	0.636	16.275	3	28	0.000
2	0.843[b]	0.711	0.668	0.7966	0.075	7.021	1	27	0.013
a. Predictors: (Constant), eCrCl V3(mL/min), V2 SUA6hr, Oxi_AUC (0-6) hr									
b. Predictors: (Constant), eCrCl V3(mL/min), V2 SUA6hr, Oxi_AUC (0-6) hr, SLC22A12_ rs505802									

Table 5:8 Uric acid disposition genes included in the analyses of GOUT-H study				
Gene	**Chromosome**	**Protein**	**Function**	**Reference**
SLC22A12	11q13.1	URAT1	Uric acid reabsorption	80,191,205,263
ABCG2	4q22.1	BCRP	Uric acid secretion	86,165,208,264
SLC2A9	4p16.1	GLUT9	Uric acid reabsorption	80,190,265
PDZK1	1q21.1	PDZK1	Scaffolding protein	80
SLC22A11	11q13.1	OAT4	Uric Acid reabsorption	80,85
SLC17A1	6p22.2	NPT1	Uric acid secretion	80,204,205
SLC16A9	10q21.2	MCT9	Uric acid reabsorption	
XDH	2p23.1	Xanthine Dehydrogenase	Uric acid production and allopurinol metabolism	266
AOX1	2q33.1	Aldehyde Oxidase1	Uric acid production and allopurinol metabolism	266
MOCOS	18q12.2	Molybdenum Cofactor Sulfurase	XDH and AOX1 cofactor	266
LRRC16A	6p22.2	CARMIL1	Actin capping protein inhibitor	80,209
GCKR	2p23.3	Glucokinase Regulator	Glucose metabolism	80,193,205

Table 5:9 SNP frequency comparisons of serum uric acid transporter genes						
Gene (SNP)	**Genotype**	**GOUT-H Study**		**Hmong Reference Cohort[79]**		***P*-value**
		N	%	N	%	
SLC2A9	CC	2	5.9%	16	7.1%	0.115
(rs1014290)	CT	10	29.4%	107	47.1%	
	TT	22	64.7%	104	45.8%	
	Total	34		227		
SLC2A9	AA	6	17.6%	77	33.9%	0.164
(rs3733591)	GA	21	61.8%	112	49.3%	
	GG	7	20.6%	38	16.7%	
	Total	34		227		
SLC16A9	GG	5	14.7%	55	23.8%	0.236
(rs2242206)	GT	22	64.7%	114	49.4%	
	TT	7	20.6%	62	26.8%	
	Total	34		231		
SLC17A1	AA	1	2.9%	6	2.6%	0.272
(rs1183201)	AT	5	14.7%	61	26.6%	
	TT	28	82.4%	162	70.7%	
	Total	34		229		
SLC22A11	AA	32	94.1%	202	87.8%	0.471
(rs173007410)	AG	2	5.9%	27	11.7%	
	GG	0	0%	1	0.4%	
	Total	34		230		
SLC22A12	CC	15	44.1%	100	44.1%	1.00
(rs505802)	CT	14	41.2%	93	41.0%	
	TT	5	14.7%	34	15.0%	
	Total	34		227		
ABCG2	AA	6	17.6%	64	27.9%	
(rs2231137)	AG	16	47.1%	119	52.0%	0.110
	GG	12	35.3%	46	20.1%	
	Total	34		229		
ABCG2	AA	8	24.2%	32	13.9%	0.266
(rs2231142)	CA	14	42.4%	101	43.7%	
	CC	11	33.3%	98	42.4%	
	Total	33		231		
ABCG2	GG	27	79.4%	181	78.4%	1.00
(rs2725220)	CG	7	20.6%	47	20.3%	
	CC	0	0%	3	1.3%	
	Total	34		231		

P<0.05 for statistical significance

Table 5:10 SNP frequency comparisons of serum uric acid non-transporter genes						
Gene (SNP)	**Genotype**	**GOUT-H Study**		**Hmong Reference Cohort[79]**		***P*-value**
		N	%	N	%	
PDZK1 (rs12129861)	AA AG GG Total	7 7 19 33*	21.2 21.2 57.6	27 97 105 229	11.8 42.4 45.9	**0.048**
XDH (rs4407290)	GA GG Total	2 32 34*	5.9 94.1	9 218 227*	4.0 96.0	0.641
AOX1 (rs3731722)	TC TT Total	4 30 34*	11.8 88.2	21 208 229*	9.2 90.8	0.544
MOCOS (rs594445)	AA CA CC Total	1 7 26 34	2.9 20.6 76.5	5 36 187 228	2.2 15.8 82.0	0.495
LRRC16A (rs742132)	AA GA GG Total	19 13 2 34	55.9 38.2 5.9	171 55 4 230	74.3 23.9 1.7	**0.041**
GCKR (rs780094)	CC TC TT Total	12 16 6 34	35.3 47.1 17.6	117 96 18 231	50.6 41.6 7.8	0.089

* Indicates SNP was not in a Hardy-Weinberg equilibrium
P<0.05 for statistical significance

Chapter 6

Concluding Summary and Futures Direction

Overall Conclusions:

The Minnesota Hmong community is a migrant minority group that came from the southern parts of China, Laos, and Thailand after becoming allies with the US during the Vietnam war. The Minnesota Hmong presents with a differential prevalence of select health conditions than non-Hmong pressing clear health disparity concerns within the community. The Hmong population is an ideal population for genetic-based studies due to their apparently low admixing rates and high cultural identity due to their strong social norms and highly structured clan system. Additionally, the high prevalence of certain health conditions in the Hmong community further augments the opportunity to identify genetic signatures or patterns associated with the manifestation of those health conditions. This expanded knowledge of disease-gene relationship can serve as a proof of concept for study designs that can be applied to other populations and ultimately addressing such health disparities in this unique populations.

The Hmong in Minnesota present with a differential prevalence of some health conditions relative to the average US population, particularly hyperuricemia and gout, as explored in this dissertation. While the prevalence of these health conditions can be partly due to a genetic predisposition for these conditions cannot be ignored. Some of these factors could be related to cultural concepts of health, perception of illness, concepts about disease management, prior reactions to Western medicine, religious beliefs, such as reincarnation, and historical immigration to the US as refugees, which may all play roles in affecting disease rates and influencing people's behaviors towards Western biomedicine. As these layers of social and cultural determinants of health affect the

Hmong residing in Minnesota, research conducted within this unique community involves careful and delicate design to account for such factors that are as important as understanding the genetics and non-genetic factors that increase their risk for these conditions.

Community-based participatory research (CBPR) model serves as an ideal approach to addressing pressing health needs in the Hmong community, identifying means to reduce health disparities and strengthening the partnership between the Hmong community and researchers at the University of Minnesota. Moreover, this approach empowers the community to influence the trajectory of the research track within the community while providing guidance on the cultural appropriateness of the study design and engaging community stakeholders to ensure the inclusivity of the community at large. Applying the principles of CBPR and guided by an established Hmong Gout Research Board, the Hmong community identified gout as a health condition worthy of study due to its significant impact on their community. In fact, the Hmong do not only have a significantly higher prevalence of gout than non-Hmong but also present with the more severe forms of gout and more gout comorbidities. The impression by members of the community is confirmed with studies that reported the high prevalence of gout in Hmong compared to non-Hmong (6.5 vs 2.9%) and especially Hmong men compared to non-Hmong counterparts (11.5 vs 4.1%). Thus, they expressed interest in collaborating with researchers to better understand what makes them different and how we may collaborate to help reduce patient suffering from this condition.

Collectively, the national and global prevalence of gout is trending upward, which

may be in part due to trends such as an increase in life expectancy, higher global consumption of alcohol and fructose corn syrup and other factors. In addition, trends identifying an increased prevalence of obesity and metabolic syndrome which appear to be associated with increased incidence of gout. While select drugs, diet, and lifestyle play a major role in the development of hyperuricemia and gout, genetic variations in the pathway of uric acid production and disposition substantially contribute to these conditions, which explain their high degree of heritability.

Genetic explorations of the prevalence of risk alleles associated with certain diseases and drug responses in the Hmong have allowed us to conclude that the Hmong are a unique population that is genetically distinct from other Asian groups while being radically different from Caucasians. In earlier chapters, we document that the Hmong to have a higher prevalence of the risk alleles associated with hyperuricemia and gout as well as different allele frequencies of clinically established drug-gene pairs. The observations of hyperuricemia and gout risk alleles in the Hmong parallel the documented higher prevalence of these two conditions in the Hmong community than the rest of the US population. Additionally, our genetic analysis results further distinguish the Hmong from Caucasians and highlights a distinction between the Hmong (who are generically described as Chinese descendants) and the Han-Chinese, the most concordant racial and ethnic group to the Hmong.

Studying special populations through clinical research is critical to the advancement of precision medicine and reaching the goal of tailored therapy to optimize treatment outcomes. Engaging the Hmong in genetic and pharmacogenetic-based studies

is a key first step to not only advance the precision medicine initiative but also provide the means to reduce, if not close, the health disparities gaps (disease prevalence, access to healthcare, healthcare literacy, healthcare quality) between the Hmong and non-Hmong in Minnesota.

In general, the response to drugs is marked with a substantial intra and inter-patient variability. While important sources of variability in response to drugs could be affected by the individual's lifestyle (smoking, alcohol consumption, physical activity, dietary restriction), concomitant use of other drugs and existing health conditions, it is well characterized that genetic factors may also play a significant role in this variability. For example, response to clinically relevant drug therapies can be significantly affected by an individual's genotype for select genes affecting its disposition and elimination. Consequently, the absence of pharmacogenetic knowledge about the Hmong presents as a healthy disparity of knowledge that could lead to sub-optimal management of their health conditions or super-therapeutic dosing that could lead to adverse events. Of course, this would be true only if there were substantive differences between the Hmong and other populations for allele frequencies of very important pharmacogenes. Indeed, this appears to be the case in Minnesota Hmong. Preliminary assessment of the pharmacogenes in the Hmong suggests the Hmong present as a distinct patient population that would require different dosing requirements for the drug warfarin relative to Caucasians based on CYP2C9 and VKORC1. The genetic variations within the warfarin response genes in the Hmong are clearly distinct from Caucasians and indicate that some Hmong patients may well require a lower starting dose than Caucasians counterparts. Additionally, the

prevalence of the CYP2C19*2/*3 indicates that approximately 58% of the Hmong would not be candidate for using clopidogrel. Collectively, the absence of this information can negatively and disproportionally widen the gap of health disparities between the Hmong and non-Hmong.

The Genetics Of HyperUricemia Therapy in Hmong (GOUT-H) study represents the epitome of a partnership between the Minnesota Hmong community and University of Minnesota researchers to address a community-identified health disparity in the Hmong community. It also provides a proof of concept for the feasibility of conducting a pharmacogenetic-based pilot study involving a culturally unique population that is minimally represented in clinical and genetic research using CBPR. Using the candidate gene approach, GOUT-H provides proof of concept for the role of genetic polymorphisms in the pathway of uric acid disposition. Not only do these key genes predict the risk of hyperuricemia and gout, but also the therapeutic response to allopurinol, which is extensively used to treat both conditions.

We have shown that allopurinol is an effective urate-lowering therapy in the Hmong with hyperuricemia or gout. Using comparable allopurinol dosing, the Hmong had a higher average of serum uric acid reduction (41%) than other reports within other populations (32%), despite achieving lower serum oxipurinol levels compared to the suggested therapeutic levels. This observation highlights the role of genetic polymorphism in the URAT1 gene (SLC22A12) transporter, which is a major transporter for oxipurinol reabsorption back to into circulation. This further supports the contribution of the URAT1 gene as it does not only modulate the risk for developing hyperuricemia and gout, but it

also serves as a marker for the response to allopurinol. Evidently, the work conducted on determining the therapeutic range of oxipurinol levels to achieve the target serum uric acid did account for the genetic polymorphisms in the URAT1 transporter gene, which explains higher serum uric acid reduction despite lower serum oxipurinol. Furthermore, the populations studied for determining optimal oxipurinol levels were mostly Caucasians who are known to have a higher prevalence of the T allele for the rs505802 T>C within the SLC22A12, which is associated with lower exposure to oxipurinol. This validates our central hypothesis of the C allele for rs505802 T>C within the SLC22A12 is associated with different oxipurinol pharmacokinetics (PK) and pharmacodynamics (PD) than the T allele. This is consistent with our findings of individuals of the TT genotype to have the oxipurinol AUC and lowest reduction of serum uric acid but the highest renal clearance of the oxipurinol compared with the CT and CC genotype.

Although the GOUT-H study provided a genetic-based evidence to the differential response to allopurinol, the relatively short duration of our study (2 weeks of therapy) precludes us from making solid predictions regarding the long-term outcomes of allopurinol therapy across the different genotypes of patients with the rs505802 T>C. However, we believe that achieving the target serum uric acid < 6mg/dL is a strong predictor of positive treatment outcomes and based on our short-term observations, the likelihood is high that it may be maintained.

Finally, one of the major barriers to achieving optimal management of gout across all populations is the patients' potential lake of adequate understanding of the pathogenesis of gout and need for chronicity of uric acid lowering therapy to prevent

subsequent gout attacks. Failure to comprehend that vulnerable patients are chronically at risk for hyperuricemia and subsequent acute gout attacks unless changes in lifestyle and diet are implemented as well as chronically taking their urate lowering drugs will render gout a sub-optimally controlled disease. Specifically, many Hmong and non-Hmong patients with gout do not fully appreciate the importance of preventative steps to avoid acute gout attacks. Rather, patients with gout not taking chronic preventive medicine often become desperate and focused on resolution of acute gout attacks rather than preventative urate-lowering therapies. In doing so, they tend to use different analgesic drugs, OTC items, dietary supplements, or alternative medicine products that promise to "naturally alleviate" their pain. In fact, some GOUT-H Hmong participants reported they would rather deal with the acute gout attack than taking a drug daily, especially when they are asymptomatic. This was discovered to be in part, due to the negative perception that using a long-term medication could damage their bodies, especially their kidneys. Dispelling such a belief plays a significant role in effectively managing gout in this community.

Hesitancy to use chronic medications out of fear that they may cause kidney damage further complicates the management of gout in the Hmong population. Therefore, education on management of chronic diseases is critical and may, in fact, change the adherence to patient's medications or study drug in our example. In the GOUT-H study, we implemented different techniques to help adherence and educate patients on the role of the chronic use of allopurinol vs. acutely to better manage their gout. These techniques included a medical presentation about gout by a Hmong primary care provider, targeted focus groups on the perception and management of gout and delivering patient reminders,

all of which may explain the high mean adherence rate observed in our GOUT-H study.

Future directions

Patient and physician education remain an area for a substantial improvement. From a primary care perspective, many physicians are apprehensive to initiate urate-lowering therapies in patients who have experienced their first gout attack. In fact, most patients are only started on chronic urate-lowering therapies by their second or third experience of a gout attack. Patient and clinician education about the optimal management of gout is needed. This is particularly true as gout often co-presents with multiple comorbidities such as hypertension, declining kidney function and metabolic syndrome all of which substantially contribute to morbidity and mortality if sub-optimally treated. Therefore, a study to compare the effect of early vs. delayed sustained initiation of urate-lowering therapy on the long-term gout treatment outcomes is warranted.

Gout is generally preceded by chronic hyperuricemia. However, treatment of asymptomatic hyperuricemia is not done clinically. Nonetheless, hyperuricemia has been shown to result in multiple comorbidities such as hypertension, chronic kidney disease, obesity and metabolic syndrome. Moreover, lowering serum acid in pre-hypertension adolescents using allopurinol 200mg twice daily has shown to significantly reduce systolic and diastolic blood pressure. Similarly, we noted a mild improvement in kidney function following 2 weeks of allopurinol therapy. These reports are suggesting that reducing serum uric below saturation levels (< 6.8mg/dL) can reap health benefits in some high-risk patients. Therefore, a long-term prospective randomized controlled trial can provide the answer to the risk/benefit ratio of treating individuals with serum uric acid higher above

saturation levels.

Allopurinol remains the mainstay for the treatment of gout though it has been associated with rare but severe allergic reactions such as severe cutaneous adverse reactions (SCAR) or Steve-Johnson Syndrome. Although these reactions have been strongly linked to genetic polymorphisms within the HLA-B58:01, preemptive testing for this polymorphism is rarely done especially in populations with such low allele frequencies. Furthermore, some reports have also linked the starting dose of allopurinol and reduced kidney function as significant covariates in for the development of these adverse reactions. From the GOUT-H study, specifically, the role of the genetic polymorphism of rs505802T>C in the SLC22A12 as oxipurinol exposure modulator, suggest that URAT1 genotype may contribute to the overall risk of SCAR. This may represent a plausible hypothesis for SCAR risk stratification as “elevated or lowered” in individuals with different genotypes, which are significantly associated with oxipurinol exposure, a major predictor for SCAR.

GOUT-H was conducted prospectively using a candidate gene approach and its effect on allopurinol response in the Hmong, however, others have used the GWAS approach to identify genetic variations associated with response to allopurinol mainly in Caucasians and Hispanics. While the nonsynonymous rs2231142 *ABCG2* A>C (Q141K) was found to associated with response to allopurinol, the same group failed to provide a physiological or in-vitro model to support this association and did not provide any PK information to support their findings. GOUT-H study, however, is the first to investigate the role of rs505802 T>C within the *SLC22A12* on oxipurinol PK and PD. This effect

needs to be validated in different population cohorts to tease out other population-specific genetics from the true effect of rs505802 T>C on the response to allopurinol. Ultimately, these validations will provide the evidence to whether this SNP could serve as a genetic marker for the selection of allopurinol in patients with gout.

While GOUT-H showed PK and PD differences in the response to allopurinol based on the *SLC22A12* genotype for the rs505802 T>C, a long-term study about the effect of those differences on the management of gout is needed. This could address the question of whether genetic-based differences in response to allopurinol are also associated with different patient treatment outcomes, such as frequency of gout flares and new onset of comorbidities that are commonly associated with long-standing hyperuricemia and gout.

Although the assessment of the effect of Hmong diet on the high prevalence of gout in the Hmong is lacking, some reports have shown that the traditional Hmong diet may be considered as uric acid-prone diet. A systematic evaluation of the typical Hmong diet in the context of hyperuricemia and gout is critical in assessing the contribution of diet and lifestyle to the overall prevalence of gout in this unique population. This assessment will also help address the interaction between the high prevalence of hyperuricemia and gout risk alleles in the Hmong and their respective diet.

Finally, a comparative efficacy assessment between the most commonly used urate-lowering therapies based on genetics and gout phenotype in the Hmong community will help advance the precision medicine of hyperuricemia and gout for the Hmong, as well as advance our approach for managing a very long known heath condition in other

populations. The impact of these present and future research endeavors will ultimately help the Minnesota Hmong reach their full potential as an integral of the community, reduce burden of health disparity on the society and improve the health wellness of the public at large.

Chapter 7

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Chapter 8

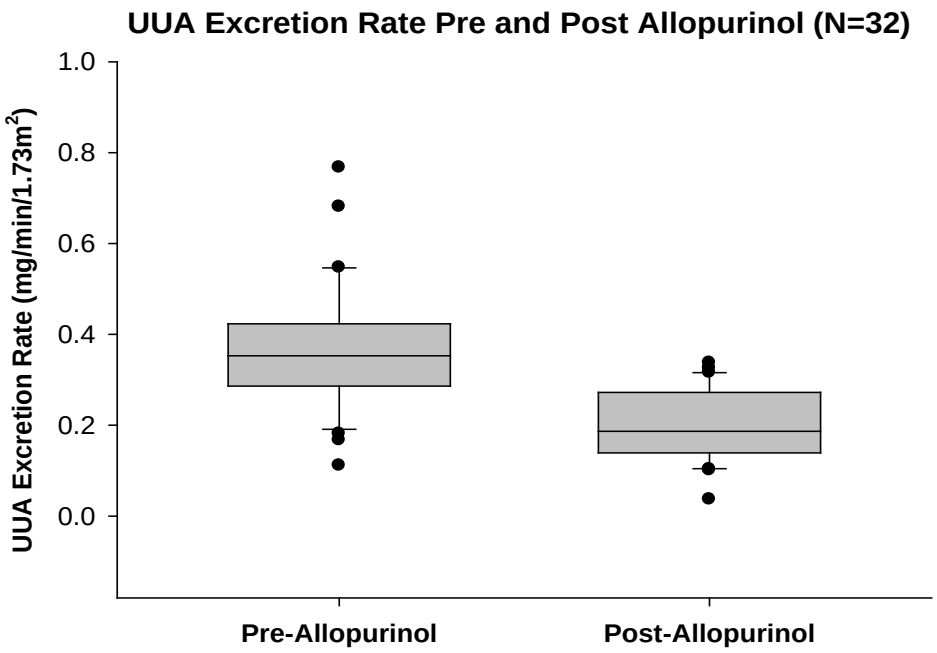
Appendix

Criteria for the diagnosis of gout [267]	
I	Monosodium urate crystals identified in joint fluid, or
II	Tophus containing monosodium urate crystals, or
III	Any 6 of the following 12 criteria:
	1. more than one attack of acute arthritis 2. maximal inflammation developed within 1 day 3. mono-arthritis 4. redness observed over joint 5. first metatarso-phalangeal joint painful or swollen 6. unilateral first metatarso -phalangeal joint attack 7. unilateral tarsal joint attack 8. suspected tophus 9. hyperuricemia 10. asymmetric swelling of joint on X-ray 11. subcortical cysts without erosion on X-ray 12. joint fluid culture negative for organisms

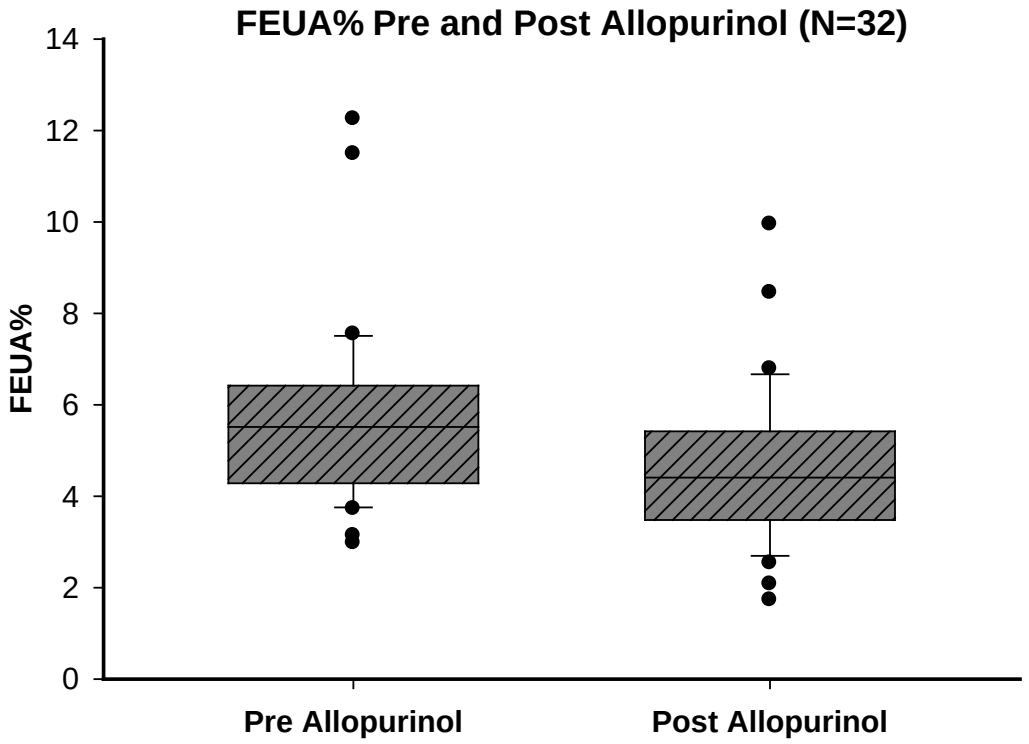
Uric acid parameters pre-and post-allopurinol therapy (N=32)			
Parameter	Pre-Allopurinol (V2) (0-6) hr	Post-Allopurinol (V3) (0-6) hr	P-value
	Mean ± (SD)	Mean ± (SD)	
UUE Rate (mg/min/1.73 m^2)	0.37 ± (0.14)	0.20 ± (0.08)	<0.0001
UCrE Rate (mg/min/1.73 m^2)	0.80 ± (0.21)	0.82 ± (0.26)	0.419
$CL_{R(UA)}$ (mL/min)	6.94 ± (2.45)	6.13 ± (2.10)	0.078
FEUA %	5.75 ± (2.03)	4.63 ± (1.73)	<0.0001
Urine Spot Ratio (U_{UA}/U_{Cr})	0.50 ± (0.16)	0.26 ± (0.13)	<0.0001

This table summarizes the analysis of only 32 participants of GOUT-H study after excluding the two participants who were taking losartan and indomethacin. As shown in the table, the renal clearance of acid is no longer significant between pre-and post-allopurinol. See table 5.3 for comparison.

UUE= Urinary Uric Acid Excretion
UCrE= Urinary Creatinine Excretion
FEUA= Fractional Excretion of Uric Acid
$CL_{R(UA)}$= Uric Acid Renal Clearance
U_{UA}= Urine Uric Acid
U_{Cr}= Urine Creatinine



These box plots summarize the distribution urinary uric acid excretion of only 32 participants of GOUT-H study after excluding the two participants who were taking losartan and indomethacin. See figure 5.9 for comparison



These box plots summarize the distribution fractional excretion of uric acid of only 32 participants of GOUT-H study after excluding the two participants who were taking losartan and indomethacin. See Figure 5.11 for comparison.

	Oxipurinol Concentration 0-hr	
	<10mg/L (n=13)	≥10mg/L (n=20)
SUA Absolute change (mean ± SD)	3.3 (1.2) mg/dL	4.6 (1.4) mg/dL*
% SUA reduction (mean ± SD)	36.2 (10.0) %	44.9 (11.7) %
Genotype counts	5 TT, 6 CT, 2 CC	8 CT, 12 CC*
N (%) with SUA < 6mg/dL	7 (54%)	16 (80%)
	Oxipurinol Concentration 6-hr	
	<15 mg/L (n=22)	≥ 15 mg/L (n=11)
SUA Absolute change (mean ± SD)	3.6 (1.4) mg/dL	5.1 (1.1) mg/dL*
% SUA reduction (mean ± SD)	36.9 (10.4) %	50.5 (8.4) %
Genotype counts	5 TT, 11 CT, 6 CC	3 CT, 8 CC*
N (%) with SUA < 6mg/dL	12 (55%)	10 (91%)

* Indicates statistical significance $p<0.05$

Oxipurinol Parameter (mean ± SD)	Participants with SUA < 6mg/dL (V3) (n= 23)	Participants with SUA ≥ 6mg/dL (V3) (n= 10)
$AUC_{0\text{-}6hr}$ (mg*hr/L)	94 (36)	67 (23)
0-hr Concentration (mg/L)	13.7 (6.3)	9.4 (3.7)
6-hr Concentration (mg/L)	15.3 (6.1)	11.1 (3.6)

SLC22A12 (rs505802T>C)	Participants with SUA < 6mg/dL (V3) (n= 23)	Participants with SUA ≥ 6mg/dL (V3) (n= 10)
TT	2 (9%)	3 (30%)
CT	11 (48%)	3 (30%)
CC	10 (44)	4 (40%)

Fisher's exact test with p= 0.37

Absolute SUA Change Stepwise Regression Models beta-coefficients*						
Model		Unstandardized Coefficients		Standardized Coefficients	t-statistic	Significance
		Beta	Std. Error	Beta		
1	(Constant)	-3.326	1.286		-2.587	.015
	V2 SUA6hr	.335	.121	.340	2.769	.010
	Oxi_AUC (0-6) hr	.031	.006	.784	4.819	.000
	CrCl V3(ml/mi)	.019	.008	.357	2.267	.031
2	(Constant)	-4.828	1.297		-3.722	.001
	V2 SUA6hr	.342	.110	.348	3.117	.004
	Oxi_AUC (0-6) hr	.040	.007	1.011	5.925	.000
	CrCl V3(ml/mi)	.021	.008	.402	2.797	.009
	SLC22A12_ rs505802T>**C**	.680	.257	.341	2.650	.013
*Dependent Variable: SUA6hr (V2-V3)						